EDITOR
DR. YAKUP GÜLEKÇİ

INNOVATIVE APPROACHES IN FORENSIC SCIENCE: SCIENTIFIC METHODS, TECHNOLOGY, AND EVIDENCE MANAGEMENT

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Published by

BZT TURAN PUBLISHINGHOUSE

Certificate Number: 202401

Delaware, United States

and Baku, Azerbaijan

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Language: English

Publication Date: December 2024

Cover designed by Kağan SARGI

Print and digital versions typeset by BZT TURAN Media Co. Ltd.

E-ISBN: **978-9952-8541-4-5**

DOI: https://doi.org/10.30546/19023.978-9952-8541-9-0.2024.211.

Suggested Citiation:

Gülekçi, Y. (2024). INNOVATIVE APPROACHES IN FORENSIC SCIENCE: SCIENTIFIC METHODS, TECHNOLOGY, AND EVIDENCE MANAGEMENT. BZT TURAN PUBLISHING HOUSE.

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Editör

Uzun yıllar olay yeri inceleme, parmak izi geliştirme laboratuvarı ve sualtı olay yeri inceleme alanlarında Emniyet Genel Müdürlüğü / Polis Kriminal Daire Başkalığı bünyesinde uzman olarak çalışan, başta “kaşıkçı” cinayeti olmak üzere pek çok dikkat çeken önemli olaylarda bilirkişilik yapan ve Kütahya Sağlık Bilimleri Üniversitesi, Adli Bilimler bölümünde öğretim üyeliğine devam eden Yakup GÜLEKÇİ, 2009-2020 yılları arasında İstanbul Emniyet Müdürlüğü'nde görev yaptı. İstanbul Olay Yeri İnceleme ekiplerinde ve İstanbul Adli Polis Laboratuvarında çalıştığı süre içinde parmak izi geliştirme yöntemleri, kan lekesi model analizi ve atışın yeniden yapılandırılması konularında geniş tecrübeye sahip oldu ve uzmanlık eğitimlerinde eğitmen olarak görev yaptı.

Lisans eğitimini 2007 yılında eğitim fakültesinde, kriminal uzmanlık eğitimlerini (olay yeri inceleme ve parmak izi arastırmaları vb.) 2012 yılında polis akademisinde tamamladı. Yüksek lisansını 2012 yılında, doktoranı ise 2017 yılında İstanbul Üniversitesi Adli Bilimler Enstitüsü'nde tamamladı. Yüksek lisans tezi "Sualtı Olgu Çalışmasından Elde Edilen Parmak İzi Delillerinin Modelleme İle Değerlendirilmesi", doktora tezi ise "Olay Yerinde Elde Edilen Patlama ve Molotof Kokteyli Atma Olayında Kullanılan El Yapımı Yangın Söndürücüler Üzerindeki Parmak İzlerinin ve Biyolojik Bulguların İncelenmesi" konularında yaptı.

Uluslararası hakemli dergilerde yayımlanan 6 adet makalesi vardır. Uluslararası bilimsel toplantılarda sunulan ve bildiri kitaplarında basılan 32 adet bildirisi vardır. 3 (üç) adet kitap editörlüğü, 6 (altı) adet kitap bölümü vardır. Ulusal hakemli dergilerde yayımlanan 10 adet makalesi vardır. İki adet dergide hakemlik yapmıştır. Bir adet sempozyum, 3 adet ulusal ve uluslararası Kongrenin bilim ve düzenleme kurullarında gören yapmıştır.

Gülekçi, çalışmalarımla adli bilimleri günlük hayatta, adli olayların çözümünde kullanılabilecek araçlara dönüştürmeye çalışmaktadır. Ayrıca; olay yerlerinde kullanılabilecek parmak izi iyileştirme yöntemleri geliştirmeye çalışıyor. Bu konuda bir patent çalışması mevcuttur.

Preface

Forensic science is an interdisciplinary field that plays a crucial role in the pursuit of truth, a fundamental requirement of justice. Rapid advancements in science and technology have introduced significant innovations in methods and techniques for solving crimes. The effective application of these innovations in forensic processes requires the continuous updating of educational and research practices. Consequently, adopting a multidisciplinary approach in forensic science while embracing innovative methods instead of relying solely on established knowledge is essential for introducing novel solutions to the evidence collection and analysis process.

Evidence Dynamics seeks to transcend the traditional understanding of forensic science by addressing the criminal phenomenon holistically, with a focus on innovative methods for evidence research. Every stage of the process from the meticulous collection and preservation of crime scene findings to their analysis through the latest technologies plays a critical role in ensuring scientific accuracy. This book thoroughly examines how new techniques and analytical methods in criminal investigations contribute to solving crimes and explores which innovative approaches can enhance the reliability of evidence.

The book does not limit itself to classical methods in evidence management but also emphasizes recent innovations, such as advanced analytical techniques and the application of artificial intelligence in forensic science. These methods not only accelerate the evidence evaluation process but also stand out as powerful tools that help ensure the proper administration of justice.

Designed primarily for students of forensic science, judges, prosecutors, and experts working in the criminal divisions of law enforcement agencies, this work provides a clear, accessible, and practical guide to evidence management. By translating theoretical knowledge into practical application, it offers a roadmap for how innovative approaches can be integrated into criminal investigations. The use of visual aids further supports the explanations, simplifying complex

concepts and illustrating how evidence can be scientifically analyzed.

In conclusion, *Evidence Dynamics* introduces an innovative perspective on evidence research in crime resolution, enabling forensic science to be applied more effectively and efficiently. We hope this book will contribute both to academic research and the practical application of forensic science, fostering the adoption and dissemination of new methodologies.

We trust that this work will serve as an inspiring resource for all researchers and professionals seeking to advance their expertise in forensic science...

DR. YAKUP GÜLEKÇİ

Önsöz

Adli Bilimler, hukukun en temel gereksinimlerinden biri olan gerçeğin ortaya çıkarılmasına bilimsel katkı sağlayan, hızla gelişen bir disiplinler arası alandır. Bilim ve teknolojideki hızlı ilerlemeler, suçların çözümüne yönelik yöntem ve tekniklerde önemli yenilikleri beraberinde getirirken, bu yeniliklerin adli süreçlerde etkili bir şekilde uygulanması, eğitim ve araştırma süreçlerinin de sürekli güncellenmesini gerekli kılmaktadır. Bu nedenle, adli bilimler alanında multidisipliner bir yaklaşım geliştirmek, sadece geçmiş bilgilerle yetinmek yerine inovatif yöntemleri benimseyerek delillendirme sürecine yenilikçi çözümler sunmayı kaçınılmaz kılmaktadır.

Delillendirme Dinamikleri kitabı, klasik adli bilim anlayışının ötesine geçerek, suç olgusunu tüm yönleriyle ele almayı ve delil araştırmalarında inovatif yöntemleri merkeze koymayı amaçlamaktadır. Olay yerinden elde edilen bulguların titizlikle toplanması ve korunmasından başlayarak, bu bulguların en güncel teknolojilerle analiz edilmesine kadar geçen sürecin her aşaması, bilimsel doğruluğun sağlanması adına büyük önem taşır. Bu kitap, özellikle suç araştırmalarında kullanılan yeni tekniklerin ve analitik yöntemlerin suçun çözümüne nasıl katkı sağladığını detaylı bir biçimde ele almakta ve delillerin güvenilirliğini artırmak için hangi yenilikçi yaklaşımların kullanılabileceğini tartışmaktadır.

Kitap, delil yönetimi sürecinde sadece klasik yöntemlere değil, aynı zamanda son yıllarda gelişen analiz teknikleri ve yapay zekanın ileri düzeyde kullanımı gibi yenilikçi yöntemlere de geniş yer ayırmaktadır. Bu yöntemler, delillendirme sürecini hızlandırmanın yanı sıra, adaletin doğru şekilde tecelli etmesine katkı sağlayan güçlü araçlar olarak ön plana çıkmaktadır.

Özellikle adli bilimler eğitimi alan öğrencilere, hâkim ve savcılara, kolluk kuvvetlerinin kriminal birimlerinde çalışan uzmanlara yönelik hazırlanan bu eser, delillendirme süreçlerine dair açık, anlaşılır ve uygulamalı bir rehber olma niteliği taşımaktadır. Teorik bilgileri pratiğe dönüştürerek, suç araştırmalarında yenilikçi yaklaşımların nasıl uygulanacağına dair yol gösterici bir kaynak sunmayı amaçlamaktadır. Görsellerle desteklenen anlatım, okuyuculara karmaşık kavramları sade

bir dille açıklamakta ve delillerin bilimsel temellerle nasıl analiz edileceğini göstermektedir.

Sonuç olarak, *Delillendirme Dinamikleri,* suçların çözümünde delil araştırmalarına yenilikçi bir perspektif kazandırarak, adli bilimlerin daha verimli ve etkili bir şekilde uygulanmasına olanak sağlamaktadır. Bu kitabın, hem akademik çalışmalara hem de adli bilimlerin uygulama alanlarına katkıda bulunarak, yeni yöntemlerin benimsenmesine ve yaygınlaşmasına ışık tutması en büyük dileğimizdir.

Adli bilimler alanında ilerleme kaydetmek isteyen tüm araştırmacı ve uzmanlar için ilham verici bir kaynak olması temennisiyle…

CONTENTS

IDENTIFICATION OF NEW PSYCHOACTIVE SUBSTANCES (NPSs) IN BIOLOGICAL MATERIALS: EVIDENCE IN FORENSIC CASES

Duygu Yeşim OVAT, Melike AYDOĞDU, Serap Annette AKGÜR

Chapter 1

Identification of New Psychoactive Substances (NPSs) in Biological Materials: Evidence in Forensic Cases

DUYGU YEŞİM OVAT[1], MELİKE AYDOĞDU[2], SERAP ANNETTE AKGÜR[3]

Overview

The emergence of a vast amount of "New Psychoactive Substances (NPSs)" represents a considerable risk to public health and safety. Due to their abundance, structure and composition, NPSs pose significant challenges to substance analysis researchers and forensic toxicologists. They also pose many challenges to global drug policy. NPSs have been described as a "growing worldwide epidemic" (Shafi et al., 2020a). NPSs, commonly so-called designer or synthetic drugs, are commercially known as "legal highs", "research compounds", "herbal highs", "bath salts", "party pills", or "internet drugs" (Peacock et al., 2019; Zuba, 2014). The regulatory bodies for NPSs include "the United Nations Office on Drugs and Crime (UNODC), the European Union Drugs Agency (EUDA), the National Institute on Drug Abuse (NIDA), and the World Health Organization (WHO)". These global organizations aim to intervene at the regulatory and policy-making levels to control or completely ban a particular substance (Al-Imam & AbdulMajeed, 2017). However, agencies working on this topic have different definitions of NPSs. The most comprehensive term, NPS, is defined by the UNODC as "a new narcotic or psychotropic drug, in pure form or in preparation, that is not controlled by the United Nations drug conventions, but which may pose a public health threat comparable to that posed by substances listed in these conventions" (UNODC, 2021). NPSs may be analogues of already regulated drugs or pharmaceutical products, or

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they tend to be "new" chemicals designed to mimic the effects of, particularly their psychoactive effects, licensed medicines and/or other restricted controlled substances (Shafi et al., 2020a).

These compounds can achieve similar effects as conventional drugs or drugs that have already been synthesized and are being used in new ways. While some NPSs were first synthesized several decades ago, the term "new" refers to recently becoming commercially available chemicals. It is a phrase used to represent a group, although it does not always refer to new inventions (Hasan & Sarker, 2023).

They are synthesized by clandestine chemists in illegal laboratories to evade international and national controls, mimicking the pharmacological effects of restricted substances and altering their chemical structure to enhance the desired effect. The laboratories are mostly located in Far Eastern countries. Organized crime groups are constantly shifting toward these substances to establish black markets because of the decrease in traditional drug use by NPSs. Due to the deficiencies in the definition of NPSs, the chemical components used to produce such compounds require very small changes to make them completely legal (Hagan & Smith, 2017). With these minor modifications, an unlimited variety of substances can be designed, produced through quick and easy synthesis steps and distributed to end users. On the other hand, scientific studies are being conducted to prove that the substances seized within this rapidly changing, infinite variety of substances have evidentiary value. This section of the book aims to provide information about NPSs, their mechanisms, and their detectability in biological materials. It also aims to present current studies that present ways to use data on these substances as forensic evidence.

Historical Perspective

Today, the types of NPSs used or seized vary according to countries' substance use trends, and these substances are classified according to their different physicochemical or pharmacological characteristics. UNODC divided NPSs into subgroups as "aminoindanes, benzodiazepines, fentanyl analogues, lysergamides, nitazenes, other substances, phencyclidine-type substances, phenethylamines, phenidates, phenmetrazines,piperazines plant-based substances, synthetic

cannabinoids, synthetic cathinones and tryptamine” (United Nations Office on Drugs and Crime., 2024). The substances classified according to their effects are as follows; synthetic stimulants,cannabinoids, hallucinogens, depressants, benzodiazepines, and opioids (Shafi et al., 2020). Synthesis of non-controlled analogs of popular drugs is not new. The first morphine analogs were prepared in the 1920s. In the 1980s-1990s, many **phenethylamines** and **tryptamines** were sythesied and introduced to the market, expanding the field of so-called “designer drugs” (Zuba, 2014). **Nitazenes** were initially studied by researchers nearly 60 years ago as an alternative to morphine but were never marketed due to the potential for overdose. Nitazenes have been linked to several overdose deaths worldwide.

Mephedrone was first synthesized in China in 1929 but did not become widely available until 2003. The use of mephedrone has also increased rapidly due to its affordable price. In 2008 mephedrone was connected to several fatalities. The increase in mephedrone use meant that it had gained a new foothold in the NPS market. By 2010, mephedrone had become illegal (Hagan & Smith, 2017).

Fentanyl was developed in the 1960s as a highly potent intravenous anesthetic that is about 50 to 100 times more effective than morphine. Illicitly produced fentanyl and fentanyl analogs have been implicated in overdose deaths over the last few decades. In a 1979 study in California, fentanyl was reported to be the cause of death in two intravenous heroin users. Drug residues were present in the deaths, and recent injection sites were found on the bodies; however, interestingly, toxicology results were negative. Fifteen deaths were recorded in 1980 following these cases, and similarly, toxicology analyses showed no evidence of drug use. Because of these suspicious deaths, law enforcement officers seized the drugs and determined that the substance contained no known drugs. They identified it as **α-methyl fentanyl**, a powerful narcotic that has not undergone scientific evaluation. Between 1979 and 1988, 112 deaths related to fentanyl and/or 10 different fentanyl analogs were reported across various states in the United States. In 1988, a chemist in Pennsylvania was

reported to have manufactured and distributed **3-methyl fentanyl**, which caused numerous deaths soon after it entered the illicit drug market (Pearson et al., 2015).

The main active compound in the production of **synthetic cannabinoids** is THC. The first "classic cannabinoid" analog synthesized in Israel in 1988 was "HU-210", and this was followed by the production of cannabinoid families chemically unlike to THC have been produced, including both non-classic and aminoalkylindole. **Aminoalkylindoles** are further categorized by JWH compounds (Hagan & Smith, 2017).

In Cambodia, sassafras oil from tree roots is used to convert methylenedioxymethamphetamine (also known as ecstasy) into its precursor chemical. In 2008, tons of sassafras oil were seized and destroyed in Cambodia and Australia. Because of this destruction, **piperazines**, which mimic the effects of ecstasy, have been synthesized and produced and sold illegally. These novel compounds became common among users because they were affordable, available, had high purity, and were egal (Hagan & Smith, 2017). Abuse was first recorded in the US and Scandinavia in the end of the 1990s, but abuse has been documented in several other countries since 2000 (Elliott, 2011).

Early Warning System

Several countries and regions have embraced different strategies to monitor NPSs through research and case reports. In 1997, the EUDA established the Early Warning System (EWS) to determine the global prevalence, elimination, market stability and market continuity of NPSs, detect trends and identify new and emerging threats. With this system, threats can be identified, monitored, detected early and intervened in a timely response. It also provides important evidence to develop new policies and interventions to address existing threats. UNODC established the Early Warning Advisory (EWA) in 2013 to increase international collaboration on the detection and reporting of NPSs, not only in Europe but also globally. The scope of work has been expanded to include identifying threats to public health and safety, determining the rate of emergence of substances, demonstrating diversity and heterogeneity in terms of substance types, and identifying problems in specific regions.

In its 2024 EWA report, it published the latest information on NPSs and an analysis of more than 2800 cases submitted by toxicology laboratories in 2023. According to this report, 1245 individual NPSs were reported to the UNODC by 142 countries and territories worldwide. In recent years, the number of emerging substances has decreased significantly; 44 NPSs were reported in 2022, and 31 NPSs were reported in 2023 for the first time (United Nations Office on Drugs and Crime (UNODC) Research and Trend Analysis Branch, 2019).

Legal and Criminal Aspects

NPS definitions vary between countries and regions, depending not only on pharmacological/structural classifications but also on social and cultural perspectives. Today, the main purpose of changing the structures is to bypass the existing anti-drug laws that emerged in most countries by ratifying the two conventions on narcotic drugs (1961) and psychotropic substances (1971). According to the conventions, a certain number of compounds is controlled in the annexes. The banning of the substance detected in the preparations was similar to that of a "cat and mouse game". New derivatives appear on the market when a substance is prohibited (Zuba, 2014).

However, under the umbrella of UN agreements, many NPSs are under global surveillance. As of December 2021, UNODC EWS reported that the majority of NPS were stimulants, followed by synthetic cannabinoids and drugs with hallucinogen receptor agonists. There has also been a recent significant increase in synthetic opioids. The rapidly altering NPS landscape has led to growing concerns about the social, mental and physical health hazards associated with the uncertainty and ambiguity of their metabolic, toxic and chemical profiles (Awuchi et al., 2023).

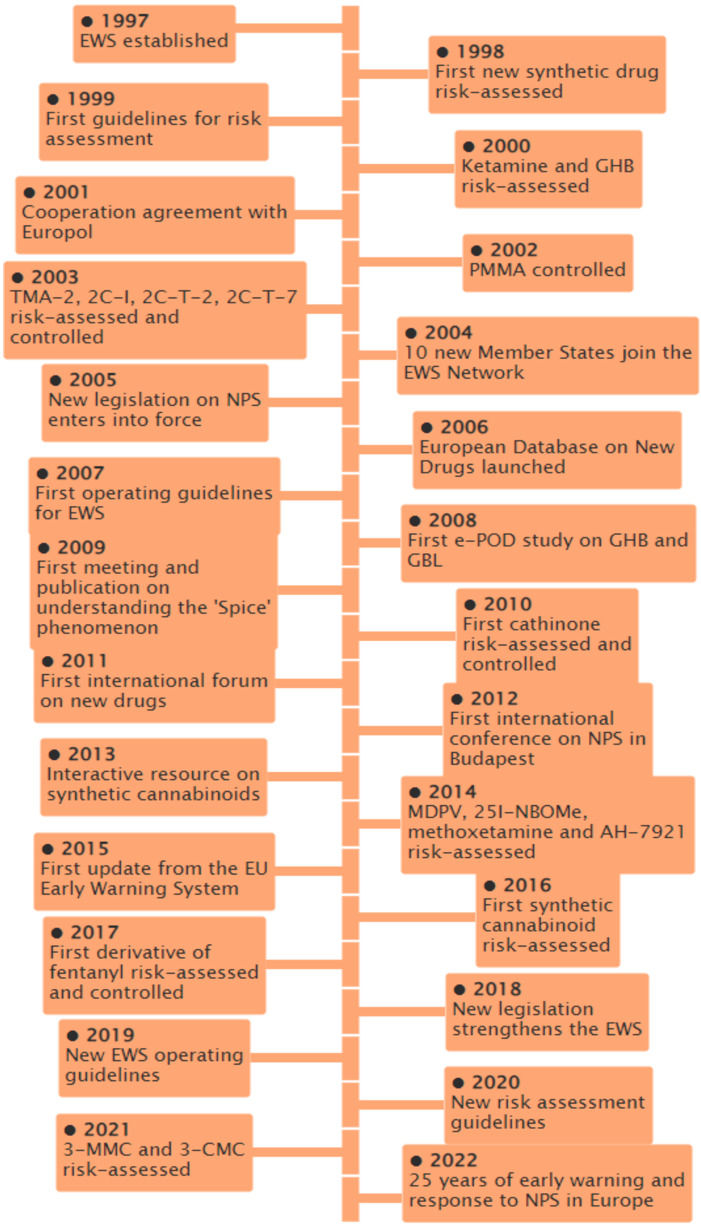

Figure 1. *Timeline of the European Early Warning System in Europe* (EU Early Warning System, 2023)

Trends in NPSs

Before 2008, the NPS market was characterized by some NPSs and a few groups of consumers, mostly from high-income countries. Accordingly, the amount, quantity, type and accessibility of NPSs have increased dramatically worldwide.

The globalization of the pharmaceutical manufacturing industries and new technologies, such as the use of the Internet, have fueled this growth market. This has enabled the industrial-scale production, supply of NPSs and the materials and supplies used to

produce, package and distribute the precursors required for production. The spread of NPSs is endemic for many different reasons, including the availability, ease of production and diversity of the chemicals used in the production phase. Today, laboratories to produce illicit substances located in the Far East are known to supply the world with vary quantities of the final products and basic chemicals required for the synthesis of NPSs. A survey by UNODC reported that these substances are trafficked by airways or by mail. Usually, when they reach European or American countries, they are repacked and redistributed by dealers via online or offline sources. The accessibility of these goods once they reach their ultimate destination is very extensive, but they basically fall into three groups: Internet, high street retailers and non-retail sellers. The Internet has become a key platform for NPS sales. The appearance and expansion of this community are becoming a concern for control agencies. High-street retailers are shops specializing in drug paraphernalia. Non-retailers are categorized as "street vendors" who source their products online or from high street retailers (Hagan & Smith, 2017).

Another problem with the production and marketting of NPS is the general instability of the products. In one study, analysis of 32 separate commercial plant materials sent to a laboratory found extreme variability in compounds, with concentrations of the common synthetic cannabinoid JWH- 018 ranging from 0.8% to >30%. Any dosage instructions can lead to inconsistent and unpredictable health outcomes. In addition to dosage uncertainty, although many NPS packages contain ingredient lists consistent with the ingredients in the products, few products have been found to contain illegal substances that are not listed on the label. This poses a great risk to users who may unknowingly consume illegal products (Hagan & Smith, 2017). In a study conducted in Türkiye in 2021, 500 urine samples that were negative for enzymatic immunoassay were selected and studied via chromatographic analysis. Of the 500 urine samples analyzed, 108 (21.6%) were reported to be positive for 20 different synthetic cannabinoids and their metabolites (Atasoy et al., 2021). Organizations

working in this field report that NPSs are present worldwide. Although numerous NPSs have been reported to date, they can vary in their nature and pharmacological effects (Tettey et al., 2018).

In general, there is little known about the potential health effects and social threats of NPSs, which poses a challenge for preventive action. Published reports on NPSs indicate that users often present to hospital with severe intoxication due to the composition and purity/impurity of NPSs. NPSs have a range of adverse effects, ranging from seizures to agitation, aggression, acute psychosis, and potential addiction. However, data on the toxicity of many NPS are not available and information on the long-term side effects and risks of these drugs is limited. (UNODC, 2024).

NPSs Prevalence

Communities use traditional methods to determine NPS use, utilizing surveys and extrapolating from reported data. These data suggest that 3% or less of the adult population reported using substances in the past year. These survey-based estimates are generally greater than estimates derived from adult populations, with some countries reporting (almost one in ten) youth having used substances recently. Some countries report a higher prevalence of NPS use compared to more traditional drugs (excluding cannabis) (United Nations Office on Drugs and Crime, 2023).

The Pharmacokinetics, Metabolism and Biological Materials for NPSs

Knowledge of the metabolism of NPSs is important for toxicological risk assessment for medical purposes and for developing toxicological analyses for forensic purposes. However, information on their metabolism is lacking to identify potential metabolic targets for analysis (Gampfer et al., 2024; Shafi et al., 2020a).

The testing of NPSs in clinical and forensic contexts can be a complicated task, as testing for such compounds in individuals presenting for drug testing is often not routinely performed, and the reliability and accuracy of existing test kits vary considerably in identifying these many novel compounds (Grafinger et al., 2020; Shafi et al., 2020a). To explain the criminal case, the analysis and detection of NPSs in

various biological materials is a complex and evolving field. This complexity arises from the rapid emergence of NPSs, which often mimic the effects of traditional controlled substances. The analysis and detection of NPSs in biological samples as forensic evidence involve sophisticated methodologies aimed at identifying and quantifying these substances, which are increasingly prevalent in the illicit drug market (Ameline et al., 2024; Richter et al., 2017; J. Tettey & Crean, 2015).

The analysis of NPSs has some significant limitations, primarily due to their various chemical structure, rapid emergence and the insufficiencies of current testing methodologies. The following sections summarize the importance/relevance of pharmacokinetics, metabolism and biological materials for the detection of NPSs in forensic science.

Pharmacokinetics and Metabolism

Pharmacokinetics and metabolism play a crucial role in forensic toxicology. Pharmacokinetics is the study of the "absorption, distribution, metabolism, and excretion (ADME)" processes of a drug. Understanding how drugs are metabolized in the body can provide important information in a variety of legal investigations, including evaluating the case in antemortem or postmortem forensic cases, determining the cause of death, assessing impairment in criminal cases, and determining drug abuse.

ADME is an important part of the study of the post-administration process of a drug molecule. This is a complex procedure comprising transporters and various metabolic enzymes with physiological outcomes on pharmacological and toxicological effects. With this process drugs are structurally modified to different metabolites. The goal is to convert drugs into metabolites that more easily excreted from the organism. The cytochrome P450s (CYPs) isozymes are primarily responsible for phase I metabolic reactions, which involve the introduction of functional groups to drugs, making them more polar and water-soluble. This process is essential for the subsequent elimination of these substances from the body. The metabolism of drugs typically involves both phase I (e.g., oxidation, reduction) and phase II (e.g., conjugation) reactions. Enzymes such as CYPs play a

crucial role in phase I metabolism, while phase II reactions often involve uridine diphosphate-glucuronosyltransferase (UGT) enzymes, which conjugate metabolites to enhance their solubility and facilitate excretion. Conjugation may also occur through acetylation or sulfoconjugation (Benoit et al., 2008; Drummer, 2008; Shafi et al., 2020b).

The liver is the main site of drug metabolism, and the first-pass effect can significantly reduce the bioavailability of orally administered drugs. This can impact the efficacy of medications and contribute to addiction by altering the levels of active compounds in the body. Excretion is the final step in drug elimination and can occur in different ways: The kidneys has an important role in excretion, especially of water-soluble drugs. Various diseases and conditions can affect kidney function. Impaired kidney function can lead to drug accumulation and toxicity. Some drugs are excreted in bile, as a parent drug or its metabolites. The bile then enters the gastrointestinal system where the drugs may be excreted in the feces or reabsorbed into the bloodstream. Small amounts of medicines can also be eliminated via saliva, sweat, breast milk and exhaled air (Negrusz & Jickells, 2008).

Genetic factors significantly influence drug metabolism. Variations in genes, such as those coding for cytochrome P450 enzymes, can lead to differences in how individuals metabolize drugs. This variability can affect the interpretation of toxicological results, especially in autopsy cases where determining the metabolic state of a deceased individual is critical (Zhou & Lauschke, 2022). Analysis of metabolites of the parent drug, can have significant legal consequences. For instance, it can help establish whether a person was under the effect of a drug at the time of an incident, which can influence charges in criminal cases. In addition, understanding the metabolism of drugs can be helpful in cases of drug-facilitated crime, where the presence of certain drugs in a convict, offender or victim system can be crucial for investigation.

Drug metabolism is essential in forensic settings as it aids in identifying the parent drugs and metabolites, understanding individual differences in drug effects, toxicity, and persistence of drugs in the body conducting forensic toxicological analyses, and influencing

legal implications. The integration of advanced techniques like metabolomics into forensic toxicology continues to enhance the accuracy and reliability of these assessments. Metabolites, which are the products of drug metabolism, can serve as biomarkers for drug intoxication or abuse. Forensic toxicologists analyze these metabolites to confirm the presence of specific drugs in biological samples, such as blood or urine. This is particularly important in cases involving new psychoactive substances, where traditional testing methods may not be effective.

Biological Materials

Forensic toxicology utilizes biological materials for analysis to investigate drug abuse or toxic substance exposure. Various biological materials are used in analytical methods for the sensitive and specific identification of NPSs, whose diversity, number and usage are increasing. The biological samples used in this field include:

Blood: Blood is the most frequently used sample for forensic toxicological analysis, particularly in postmortem investigations. It is essential for determining the presence and concentration of drugs and poisons in the body. In addition, a blood sample allows the assessment of whether a person is under the influence of a substance in antemortem cases.

Urine: Urine is another standard sample, often collected for drug screening due to its ease of collection and ability to detect recent drug use.

Hair: Hair analysis is valuable for assessing long-term exposure to drugs. It allows for the detection of substances over extended periods, making it useful in cases where historical drug use is relevant.

Nails: Similar to hair, nails can provide a record of drug exposure over time and are increasingly used in toxicological assessments.

Bile and Gastric Contents: These samples can be analyzed to determine the presence of substances ingested shortly before death, aiding in understanding the circumstances surrounding a death.

Liver and Brain Tissue: These tissues are often analyzed in postmortem examinations to ensure insights into the metabolic effects of drugs.

Alternative Matrices:

- Recent advancements have led to the use of alternative biological matrices such as:
- Oral Fluid: Useful for detecting recent drug use (Busardò et al., 2024; Marchei et al., 2024).
- Sweat: Can provide information on continuous drug exposure (Busardò et al., 2024; Gomes et al., 2024).
- Meconium: Analyzing this can indicate prenatal drug exposure (López-Rabuñal et al., 2019).
- Breast Milk: Important for assessing drug transfer to infants (Baker et al., 2023).
- Vitreous Humor: Its unique properties make it particularly suitable for detecting substances after death, is less susceptible to postmortem changes compared to blood and urine (Andrade et al., 2016; Campos et al., 2022).

Data are already existing on detection in alternative matrices for more conventional drugs (morphine, cocaine, etc.). For newly emerging drugs such as NPSs, data on detection NPSs in alternative biological matrices are limited (Campos et al., 2022).

In forensic cases involving drug abuse, both blood and urine are the most commonly used biological materials for drug detection, but they have different advantages and disadvantages. The detection window for drugs in blood is generally shorter compared to urine. This implies that blood is the better material for detecting recent drug use, typically within a few hours to a few days after ingestion. Blood tests are more invasive and expensive compared to urine tests, which can limit their use in some settings and provide a direct measure of drug concentration in the body. It allows for precise analysis and identifying of drugs and metabolites. Urine accumulates drug metabolites over time, allowing for the detection of past drug use. Urine tests have a longer detection window compared to blood. They can detect drug metabolites for several days to weeks after use, making them suitable for drug testing. Urine is non-invasive and easier to collect compared to blood and it is more susceptible to tampering and adulteration, which can lead to false-negative or false-positive results. However, urine is commonly used for routine drug screening and monitoring of substance use.

In summary, blood provides more accurate information about recent drug exposure and intoxication, while urine is better for detecting past drug use, offers a longer detection window and is more practical for routine screening with more convenient collection. Forensic drug testing often utilizes both blood and urine samples to obtain a more complete picture of an individual's drug use history and current state. As a result, it is valuable to identify NPSs together with their metabolites in human biological materials with reliable and clinically validated tests. Different techniques based on colorimetric, immunoassay or chromatographic are used in NPSs detection. It is reported that very few NPSs can be detected today, and this situation has vital importance in clinical and especially forensic processes.

Analytical Strategies

The transformation of drugs into metabolites with various chemical and physical properties as a consequence of metabolism in the body and the identification of these metabolites is very important not only for the pharmaceutical and medical field but also for providing evidence in forensic toxicology. In the last years, it is known that forensic toxicology has suffered an influx of NPSs (Drug Enforcement Administration & Control Division, 2020; European Monitoring Center for Drugs and Drug Addiction (EMCDDA), 2022; Lukić et al., 2021). Apart from traditional substances, NPSs are more commonly used for (i) *to produce equivalent psychoactive responses by targeting similar systems in the body*, (ii) *to avoid detection by using a substance that is known and likely to be detected in drug tests*, and/or (iii) *because of analytical limitations in identification* (Soussan et al., 2018). At the same time, clandestine laboratories steadily create NPSs to circumvent legal classification efforts, making toxicological analyses challenging and complex. This poses new analytical challenges in the detection of these chemically variable NPSs, in terms of the identification and classification of the substance in seized material. The metabolic fate of NPSs is not entirely predictable, however, since metabolic studies have been conducted extensively, "**NPSs profiling**" provides a significant amount of hard evidence, not just a prediction.

Emerging NPSs present a significant challenge for drug testing laboratories, as new substances cannot be identified by current analytical methods. Quantitative and robust liquid chromatography-high-resolution mass spectrometers (LC-HRMS) allow analysts to go further beyond targeted analysis (Karabulut & Ertaş, 2021). New high resolution mass spectrometers are capable of detecting large numbers of molecules (from 100 to 1000) at low levels in the same analysis. These new detectors mainly consist of HR-MS, Orbitrap and Time-Of-Flight-MS. Especially, HR-MS allows discrimination among highly similar mass spectrometers such as, $C_{15}H_{14}O_3N$ or $C_{16}H_{16}OS$ can be easily distinguished by their m/z values: 257.10464 and 257.09946 Da (delta = 5 mDa), respectively. HR-MS analysis showed excellent selectivity 'with full scan acquisition, while 1000s of ions were recorded in the LC-MS analysis″(Rochat et al., 2014).

In general, in laboratories using HR-MS, both global and non-targeted data are simply obtained, but the data are often processed in a targeted way to identify the compounds that are expected, however, when considered non-targeted, these big data can possibly reveal unexpected compounds of interest in specific samples (Rochat et al., 2014; Wu & Colby, 2016). Therefore, laboratories are challenged to improve and validate outdated methods to identify NPSs. Currently, non-targeted screening methods are often used for the identification of NPSs, based on LC-HRMS and retrospective searches of previously obtained data for the identification of NPSs (Di Trana et al., 2021; Stork et al., 2020). An untargeted screening method is beneficial for the identification of NPSs either known or unknown at the point of testing. To target the ever-changing class of substances to be identified, there is a significant endeavor to develop HR-MS screening methods that are fast with all non-targeted ion detection and can be updated more rapidly with new drugs (Diao & Huestis, 2017a; Kronstrand et al., 2014). High-resolution MSs efficiently first identify and then validate substance-specific fragment ion formulas by providing accurate mass measurement, and also enable laboratories to achieve metabolite definition with improved efficiency and quality (Marchei et al., 2018; Stork et al., 2020). Virtually the

only restriction of targeted screening methods is the *fast-changing NPS family*.

The analysis of NPSs in biological samples is compelling, especially NPSs that are rapidly metabolized at low active doses. Therefore, it is essential to investigate specific biomarkers of substance use. In these studies, metabolite profiling studies of NPS are usually performed first. Then, human and/or animal hepatocyte incubations are performed and, if possible, completed with the analysis of real human samples (Di Trana et al., 2021; Diao & Huestis, 2017b). *Urine*, a very important material in forensic toxicology, is the most widely used matrix in NPS profiling due to its sample volume, reflecting high drug concentrations and offering a longer drug detection window, especially versus to blood and oral fluid (Scheidweiler et al., 2015). NPSs can also be detected in blood and oral fluids if the laboratory has knowledge of the molecular structure and molecular weight of the substance of interest or uses a non-targeted analytical approach. However, the detection windows for these potent substances are short and therefore it is critical to target marker metabolites in urine.

The elucidation of the pharmacological and pharmacokinetic processes and the discovery of metabolism products provide important evidence. The fate of the substances, delivered directly to our bodies is mainly managed by the three phases of drug metabolism: **"phase I**, addition of a reactive group (by means such as oxidation, reduction, or hydrolysis); **phase II**, conjugation with diverse bonds; and **phase III**, elimination of drugs and metabolites from liver and intestinal cells" (Mackenzie et al., 2017). The metabolism of substances is commonly predicted based on their interactions with cytochrome P450 (CYP450) enzymes and their metabolic endpoints (Tan et al., 2017). In cases, urine samples are usually hydrolyzed under appropriate conditions using the beta-glucuronidase enzyme. As a result of this process, characteristic phase I metabolites are selected as biomarker metabolites in the urine to confirm substance use. Most psychoactive substances/drugs are excreted by phase II metabolism, as in NPSs, with the formation of glucuronide or sulfate metabolites. However, the

disadvantage of this is that the glucuronides and sulfates formed in phase II are not as stable as phase I metabolites (Scheidweiler et al., 2015; Wohlfarth et al., 2013). Therefore, strategies for the detection of possible metabolites, especially after phase I reactions, resulting from the use of a new generation substance such as NPSs, which have not been previously identified or whose newly identified metabolites are unknown, are a necessity. Multiple approaches have been used to estimate urine marker metabolites including;

-in vitro incubation in human liver microsome (HLM),

-in vitro incubation in human hepatocytes,

-in vivo application of controlled drugs studies on rats or mice and also rodents, zebrafish larvae,

-***in silico*** prediction.

Alternative approaches recommend the most abundant and typical metabolites as "marker metabolites", which can then be validated in real urine samples from suspected NPS cases. In vitro modeling for substance metabolism has to exactly mimic in vivo biotransformation. Over the last few decades various in vitro models of the human liver have been developed in the pharmaceutical industry, the most commonly utilized and well-established in vitro incubation methods are liver microsomes and hepatocytes, which are also approved by relevant officials such as "the US Food and Drug Administration" (Brandon et al., 2003). The use of human hepatocyte incubation and HR-MS to elucidate NPS metabolites is the most favored coupled approach. Hepatocytes, isolated living cells, are a physiological system that enables to simulate human metabolism with the presence of extensive phase I and phase II enzymes and drug-binding proteins (Costa et al., 2014). HLM is the most widespread *in vitro* model due to its lower expense and ease of handling. Albeit, hepatocytes present a metabolic environment more similar to liver physiology than HLM, with some drawbacks. The main benefit of hepatocytes versus HLM is that robust liver cells generate either phase I and phase II metabolites and inappropriate abundance; however, the abundance of HLM metabolites may not reflect the prevalence in real liver cells (Brandon et al., 2003; Diao & Huestis, 2017c). For that reason, metabolites can be used as

a reference for verifying metabolites found in urine.

Early estimation of metabolic pathway is crucial to pre-clinical, clinical and decision-making. For drug discovery, it is essential to have trustworthy data on exactly how a compound reacts in the presence of metabolizing enzymes. This demands considerable experimentation effort. As a consequence, the ability to estimate possible sites of metabolism in any synthesized or virtual compound will be highly useful and time-efficient (Afzelius et al., 2007; Diao & Huestis, 2017c). In silico-based applications are progressively being adopted to estimate the metabolic transformation of drugs and are therefore recognized as the best approach to predict the metabolic transformation of drugs, allowing for lower costs, timesaving and hence reduced rates of late drug discovery (Kazmi et al., 2019). To date, forensic toxicology studies investigate the metabolic pathway of substances using *in silico* predictions, human hepatocyte incubations and LC-HRMS/MS detection in an original workflow to identify relevant consumption markers. *In vitro* incubations with human hepatocytes have been shown to be appropriate for the estimation of NPS pathways in former research and LC-HRMS/MS has evolved into the gold standard method for exhaustive scanning of sophisticated materials. In addition, novel data mining based on dual-targeted/untargeted analysis is often used, allowing for fast and semi-automated characterization of NPS metabolites via "NPSs spectrum libraries" such as "mzCloud and HighResNPS". The general workflow of freely available mass spectrum databases is well suited for rapid implementation for NPS metabolite identification researches, given the market dynamics and underground production (Carlier et al., 2018). Furthermore, "metabolite samples from human hepatocytes" can be used as reference for verifying metabolites identified in urine, but their precise structure (i.e. the position of hydroxyl groups) may not be obtained by MS alone, for precise structural elucidation further techniques such as "nuclear magnetic resonance spectroscopy" may be required (Gandhi et al., 2013).

In vitro and *In silico* Practices to Provide Evidence

In this process, although the flow chart may vary according to the infrastructure of the laboratories, the purpose of the analysis and the target analyte, the workflow chart is generally carried out in the following order.

I. *In vitro* Practices

The workflow for metabolism research of NPSs is generally recommended as follows: (i) incubate NPSs in human hepatocytes; (ii) define the most abundant and typical metabolites in hepatocyte samples by HR-MS; and (iii) acquire true positive urine samples and validate hepatocyte metabolite markers.

II. *In silico* Practices

"*In silico prediction also aids metabolite definition without any reference standard* and proposes metabolic sites and potential metabolites". *In silico* software tools are widely applied for the estimation of NPSs metabolites. These software programs essentially aim at CYP450-mediated biotransformations, specifically considering phase I and phase II substance metabolizing enzymes. These are metabolites that may form through processes such as hydroxylation, acetylation, demethylation, dealkylation, and glucuronidation. *In silico* estimation is an important evidential tool for metabolite elucidation, although there are sometimes discrepancies between urinary metabolite profiles.

- **Data Mining**

Databases and related software prepared for *in silico* studies enable the prediction and ranking of metabolites via the integration of "machine learning-based" reaction estimation fields to determine reaction norms (Solimini et al., 2018). The list of metabolites is also created by related custom software. Usually, a match score is determined and metabolites higher than this score are selected. The data is re-processed to mimic the "second-generation metabolism reaction; the second-generation metabolite score is multiplied by the first-generation metabolite score, and scores higher than the current score" are considered. The specific workflow commonly used and developed for data mining is given in Figure 2.

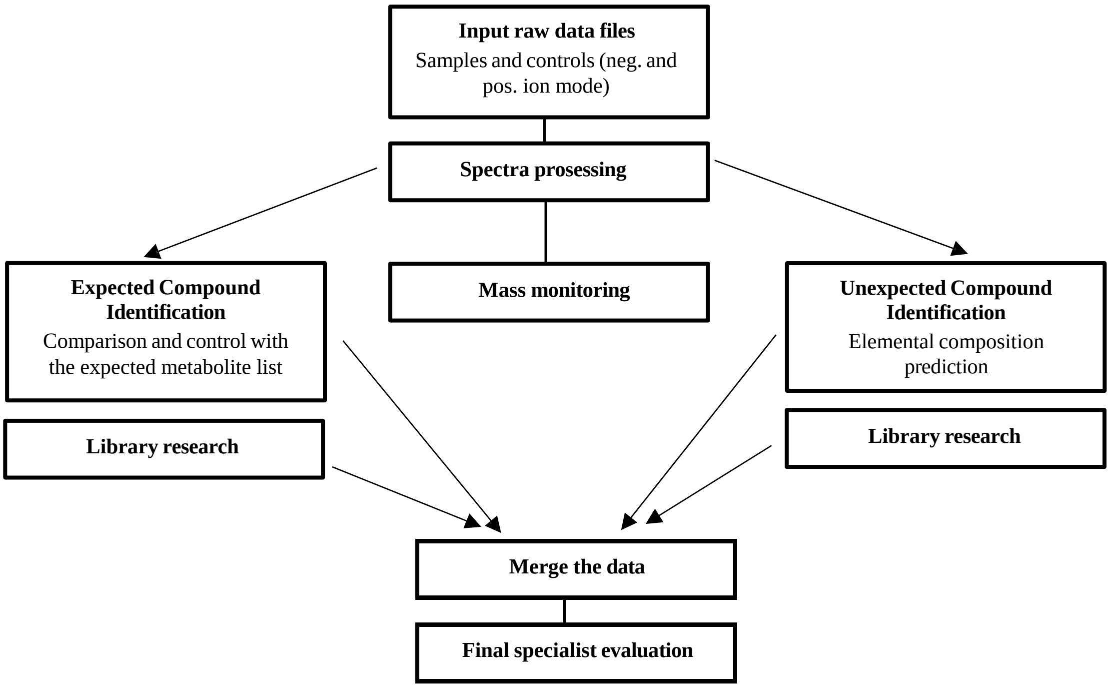

Figure 2. *Raw data mining workflow using LC-HRMS/MS*

Pre-processing: Following analysis of the "raw data of samples and controls, the retention times of peaks in the mass spectra are evaluated with a maximum shift of 0.1 min and a mass tolerance of 5 ppm". In full-scan HRMS, data are processed in the same way in both positive and negative ion selection modes.

Untargeted data mining: It is usually evaluated based on peaks with an intensity above 10^6, a signal-to-noise ratio above "3 and a 30% intensity tolerance for isotopes" (these values may vary according to the analyte) (Di Trana et al., 2021; Kazmi et al., 2019; Taoussi et al., 2024). Mass spectra and molecular formulas are then checked with chosen libraries.

Targeted data mining: For targeted data mining, a theoretical metabolite list is produced by combining probabilities. Usually, "peaks with intensity above 5×10^3, signal-to-noise ratio above 3 and intensity tolerance of 30% for isotopes, observed peaks with a mass tolerance of 5 ppm" are compared to the compound list. Compounds are finally sorted between the data files based on a retention time tolerance of 0.1 min and compared to the libraries (Gergov, 2004; Taoussi et al., 2024).

Findings from non-targeted and targeted data mining applications are combined and compounds identified in controls and with equal or greater

abundances than those detected in incubations are screened out. Outcomes are ultimately evaluated by the expert for final definition and structure clarification.

Literature review on utilize in vivo, in vitro and in silico approaches for NPS profiling

In this section, a brief literature review was conducted on studies that utilize in vivo, in vitro and in silico approaches for NPS profiling to elucidate biomarkers that may indicate the use of known or unknown target substances of interest in forensic toxicology. In this research, studies conducted in the last decade aimed at the analysis of substances from the NPS class were discussed.

Methods: The research design was prepared in accordance with the PRISMA guidelines. The findings of the systematic review are provided in the flow diagram of the literature review (Fig. 3). After the inclusion and exclusion criteria were determined by the researchers, the data obtained were evaluated separately by the authors and then cross-checked. The research was conducted using the keywords "in vivo", "in vitro", "in silico", and which were matched separately with the terms "New Psychoactive Substance" on PubMed, Wos and Scopus databases in the last decade. The search by keywords was aimed at preventing possible data loss by covering all sections, such as title, keywords, and abstract, with the "title, abstract, keywords" search option in databases. The data obtained was stored in *.xls* extension on the computers of the researchers and the data obtained from the three databases were combined. Considering the names and DOI numbers of the studies included in the study, duplicate publications were manually removed by removing duplicates in the Excel file. Following the removal of duplicate publications, the researchers evaluated the remaining publications by reviewing the abstracts in terms of content appropriateness. If the title and abstract were assessed as suitable, the study included these publications.

Inclusion Criteria: The study included research articles and case reports published in English between January 2014 and September 2024, which met the search criteria based on keywords. Research articles, reviews, books, book chapters, Letters to the editor, and Viewpoints were included.

Exclusion Criteria: Studies not written in english and Erratums were technically excluded. Studies filtered after technical screening according to the title. Studies that did not serve the purpose of the review; (i) study titles that addressed the receptor activity, behavioral effects and addiction formation of NPSs in the field of clinical toxicology and (ii) studies that did not mention the use of in vivo, in vitro or in silico methods were eliminated.

Results: A total of n=1247 studies were filtered with the keywords in three separate databases. One of these was excluded due to being an Erratum, and n=755 were eliminated from the study as duplicates. The remaining studies were excluded due to incompatibility with the designed research topic specified in items (i) and (ii) of the exclusion criteria. Studies with titles incompatible with the purpose (n=179) were eliminated. The abstracts of the remaining studies were read by two researchers. According to the abstracts of the studies that were incompatible with the objective, n=113 studies were excluded. After eliminating n=15 studies without full text access from the remaining studies, 173 were included.

Challenges in monitoring NPS use can be overcome with different analysis techniques to learn more about the prevalence and expansion of NPS use. In the literature review, 173 out of 1237 studies used *in vivo*, *in vitro* and/or *in silico* methods to detect NPSs, identify possible metabolites indicating their use and elucidate their metabolic pathways. With these alternative methods, it will be able to figure out further the pathways by which the NPS metabolism can be estimated with higher confidence. Not only will this contribute to improving better methods to manage NPS intoxication, but it will also be useful in assisting in compiling forensic and medico-legal reports for the jurisdiction.

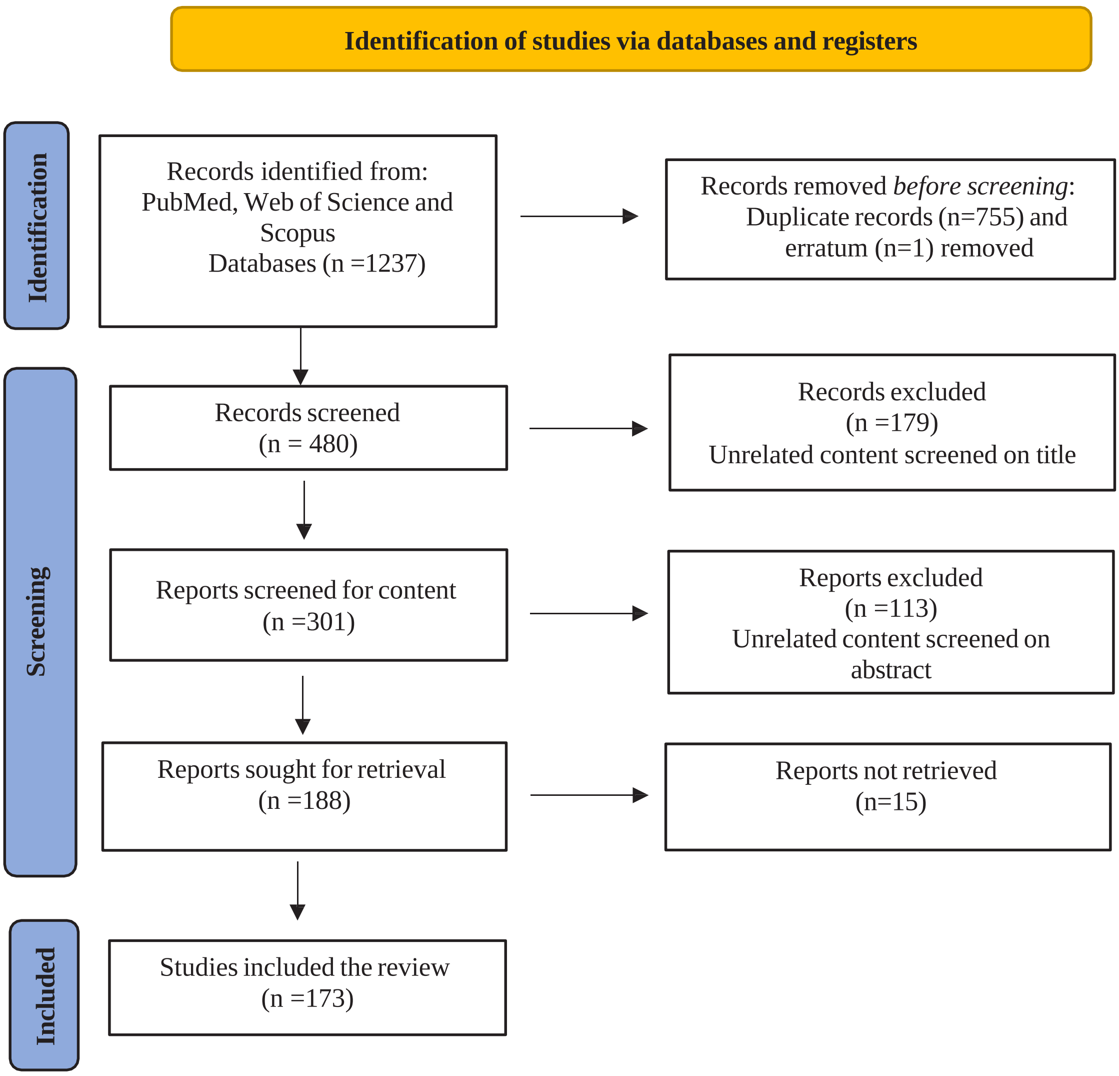

Figure 3. *Flow diagram of the literature search (Prisma flow diagram)*

Conclusion

Future Perspectives

Novel Psychoactive Substances consist of a broad variety of synthetic substances specially designed to produce psychoactive effects. They are typified by a structural multiplicity produced by altering the molecular structure of available substances or conventional ones. However, such structural diversity poses difficulties for regulatory authorities and makes it harder to keep a close monitor on the proliferation and abuse of these substances. The problem is compounded by the analytical challenges of analyzing NPSs. With the current information provided, new analytical methods other than traditional ones are presented to obtain forensic evidence to detect NPSs. Thus,

NPSs, which are increasingly being used in an uncontrolled manner and non-detection of NPSs creates forensic problems, can be prevented from posing a risk to human health and public safety.

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SKELETAL ANATOMY ANALYSIS AND EVIDENCE COLLECTION AT THE CRIME SCENE: FORENSIC ANTHROPOLOGY AND ARTIFICIAL INTELLIGENCE APPLICATIONS

Mert OCAK , Cumali Çatak

Chapter 2

Skeletal Anatomy Analysis and Evidence Collection at the Crime Scene: Forensic Anthropology and Artificial Intelligence Applications

MERT OCAK[1] , CUMALİ ÇATAK[2]

Introduction

Integrating artificial intelligence (AI) models and machine learning into forensic processes represents an effective approach to victim identification, particularly by utilizing advanced biomedical imaging techniques alongside anthropological and anatomical measurements. The most common imaging methods in forensic identification are X-rays and computed tomography, both of which provide critical information about skeletal remains and facilitate the extraction of anatomical features necessary for age estimation and sex determination. These imaging techniques are increasingly recognized for their effectiveness in forensic investigations, enabling detailed analysis of skeletal materials, especially in scenarios such as mass disasters where traditional identification methods may be inadequate (Vaswani, 2023).

The application of AI, especially through deep learning methodologies such as Convolutional Neural Networks (CNN), has revolutionized imaging data processing. CNN are adept at performing feature extraction and classification tasks necessary for automatically analyzing anatomical measurements from processed images (Nasien, 2023). Preprocessing of imaging data, including standardization of dimensions, noise reduction, and contrast enhancement, improves the learning capacity and prediction accuracy of the model. Furthermore, the application of data augmentation techniques and transfer learning has proven to be effective in improving model generalization, thereby increasing the robustness of age- and sex-related predictions (Shorten et al., 2021).

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In forensic anthropological studies, a systematic review of existing studies on structures such as the mandible, femur, and pelvis provides a rich dataset for training machine learning models (Woon & Stringer, 2012). Researchers continue to work on new models and develop methods to obtain more accurate and reliable prediction and identification results by analyzing anthropological measurements and demographic information.

The implications of using AI and machine learning in forensic sciences go beyond identification, covering the ethical and practical dimensions of evidence collection and skeletal analysis. AI tools provide a more scientific basis for forensic evidence by minimizing human error and subjectivity (Vaswani, 2023). Moreover, the fast processing capabilities of AI significantly speed up identification processes in emergencies, thus improving the effectiveness of forensic investigations (Thurzo et al., 2021). The target audience of this research is professionals and academics in the fields of forensic sciences, forensic anthropology, medicine, and AI. The research aims to increase the interest of experts and relevant stakeholders in forensic science methods integrated with evolving technology, encouraging them to contribute to the field by critically evaluating studies conducted on the subject (Baryah et. al., 2019).

The Role of Forensic Anthropology in Crime Scene Investigations

Forensic anthropology plays a crucial role in crime scene investigations, identification, and analysis of human remains. This speciality combines the principles of anthropology and forensic science to assist law enforcement in solving crimes involving unidentified bodies or skeletal remains. When soft tissues are distorted and decomposition has taken place, the skeletal structure becomes compromised and the remains fragmented. Forensic anthropologists have the expertise to extract crucial information from the evidence to determine the identity and circumstances of death. (Sharma et al., 2011; Devraj et al., 2022).

They create a biological profile by studying bones to determine sex, estimate age, and analyze trauma for unidentified individuals. This profile is then compared with antemortem

information and DNA analysis to aid in identification. Working alongside forensic pathologists and law enforcement, forensic anthropologists provide vital details about the deceased, such as age, sex and height, which are crucial for the identification process. (Kahana & Hiss, 2009; Boer et al., 2018).

Collecting evidence at crime scenes is a meticulous process that requires a thorough understanding of both anthropological techniques and forensic protocols. Forensic anthropologists specialize in recognizing and recovering skeletal remains, ensuring that the context of evidence is preserved. Lack of sufficient expertise in this area can lead to problems in cases such as fires, and the integrity of evidence can be compromised. In short, the anthropological approach to crime scene investigation emphasizes the importance of a multidisciplinary team, including forensic archaeologists, to ensure that all potential evidence is documented and recovered systematically (Porta et al., 2013; Schultz & Dupras, 2008).

In complex cases such as natural disasters, the stress experienced by crime scene investigation teams can affect their performance and the quality of evidence collected. In such situations, it is important to increase methods integrated with technological innovations that will reduce stress for crime scene investigation teams and improve the accuracy of their work (Adderley et al., 2012; Eeden et al., 2018).

Forensic anthropologists also have a major role in broader societal events, such as human rights violations along with major disasters. Forensic anthropologists use their expertise to identify victims in these cases and take their place to assist in the subsequent legal processes. Challenges such as the need for rapid response and management of large-volume remains to emphasize the critical importance of forensic anthropology in contemporary forensic sciences (Boer et al., 2018; Ferllini, 2017).

Biological Profiling

The biological profile typically includes estimates of sex, age at death, ancestry, and stature. Each of these components plays a vital role in narrowing down the identity of skeletal remains. For example, sex

determination is often prioritized due to its significant impact on the accuracy of subsequent biological assessments (Winburn & Algee-Hewitt, 2021; Belcher et al., 2021). While ancestry estimation is challenging, it is equally important as it can provide critical context about an individual's background and potential familial connections (Passalacqua et al., 2023; Boyd & Boyd, 2011).

Sex determination is one of the most critical stages of biological profiling. Traditional methods rely on morphological features of the pelvis and skull, which are known to exhibit sexual dimorphism. The pelvis is particularly useful due to its reproductive function, making it the most sexually dimorphic skeletal element (Francisco et al., 2017; Barbieri et al., 2018). Recent advancements have introduced quantitative methods, such as geometric morphometrics and three-dimensional imaging, which have increased the accuracy of sex estimation (Soriano et al., 2022; Goldstein et al., 2022). Additionally, the reliability of sex determinations has been further enhanced by using machine learning algorithms to analyze skeletal features (Górka & Mazur, 2021; Keyes, 2023).

Age estimation in forensic anthropology has become an important field, especially with studies on skeletal remains of individuals in childhood and adolescence. In this context, "non-adult" remains to cover individuals from birth to 18 years of age, and age estimation of these remains is based on factors such as tooth development, bone fusion (ossification), and skeletal maturity. In adult remains, skeletal maturity is among the most commonly used indicators. This involves assessing skeletal features such as epiphyseal fusion in long bones and the ossification of the iliac crest, clavicle, and sacrum to determine whether the individual has reached at least 18 or 20 years of age. The pubic symphysis is considered one of the most reliable methods for age estimation, especially for individuals under 40. In-situ photography of the pelvis at the crime scene, along with careful packaging, is recommended to ensure the integrity of the study. Another frequently used method is age estimation from the sternal end of the ribs developed by İşcan et al. (İşcan et al., 1984; İşcan et al., 1985; İşcan et al., 1987). Imaging methods

(conventional radiography, computed tomography (CT)) and histological analyses have recently played an important role in age estimations. Researchers have achieved successful results through the histological analysis of long bones. Molecular and chemical methods, particularly techniques like aspartic acid racemization, also show promising potential in age estimation (Crowder & Stout, 2011).

Estimating the age at death from skeletal remains involves various methodologies, including dental analysis, epiphyseal fusion, and histological examination of bone microstructure. Each method has strengths and limitations, and often, a multifaceted approach is necessary to obtain accurate results (Chatterjee et al., 2020; Nasien, 2023). For example, while tooth wear patterns can provide insight into an individual's age, the fusion of skeletal elements can indicate developmental stages (Rizos, 2023; Baryah et al., 2019). Recent studies have also explored the use of advanced imaging techniques, such as dual-energy X-ray absorptiometry, to assess age-related changes in bone density (Zhang, 2024; Bethard, 2016).

Stature estimation is another critical component of the biological profile, usually derived from long bone measurements. Various regression formulas based on population-specific data have been developed to estimate height from skeletal remains (Diac et al., 2021; Yang et al., 2020). However, the accuracy of these estimates can be affected by factors such as population diversity and environmental conditions. Recent advances in imaging technology and statistical modeling have improved the precision of height estimates, allowing for more reliable applications in forensic cases (Pilloud et al., 2022; Robles et al., 2023).

Biomedical Imaging Methods Used in Forensic Anthropology

Forensic anthropologists also use biomedical imaging techniques to analyze skeletal remains and fragments recovered from crime scenes. These techniques include X-ray and CT scans to visualize internal structures without damaging evidence, in addition to the application of classical osteometric methods (Porta et al., 2013). While these techniques help identify remains in investigations, they can also provide valuable information about the cause of

death through analysis of trauma on bones (Eeden et al., 2018).

Forensic anthropology is increasingly integrating advanced biomedical imaging methods to improve the accuracy and reliability of investigations involving human remains. The application of imaging techniques such as CT, magnetic resonance imaging (MRI), and three- dimensional (3D) surface scanning has revolutionized the field by offering non-invasive alternatives to traditional autopsy methods. These imaging methods not only facilitate the examination of skeletal remains but also allow for assessing soft tissue injuries and other pathological conditions that may not be visible through traditional methods.

CT and MRI have emerged as essential tools in forensic imaging, providing detailed information about complex body structures and allowing for the examination of highly decomposed or contaminated remains. Chen highlighted the advantages of post-mortem imaging in examining areas typically inaccessible during traditional autopsies, such as the thoracic cavity and brain. These imaging techniques can also be applied to cases involving infectious diseases or toxic substances where traditional methods may pose health risks to forensic personnel (Chen, 2017; Singh et al., 2022). Additionally, the integration of CT angiography allows for the visualization of vascular structures, which can be crucial in cases of trauma or homicide.

The application of imaging techniques extends beyond examining skeletal remains; they also play a significant role in assessing soft tissue injuries. Zhang emphasized the utility of imaging in sex estimation by analyzing cranial and pelvic structures, which can be critical in forensic investigations where biological sex is a determining factor in identification. The ability to visualize and analyze these structures non-invasively increases the accuracy of forensic assessments and reduces the potential for damaging remains.

Imaging methods contribute to diagnostic capabilities and the legal and ethical dimensions of forensic anthropology. The non-destructive nature of these techniques aligns with the increased emphasis on respecting the dignity of the deceased and the

wishes of their families, particularly in cultures where traditional autopsy methods may conflict with religious beliefs (Ahuja & Ansari, 2022). The use of imaging promotes a more respectful approach to forensic investigations, allowing forensic experts to gather necessary information while minimizing physical intervention with the remains. Integrating AI and machine learning into forensic imaging is a promising new area of research for enhancing the capabilities of forensic anthropologists. AI algorithms can analyze imaging data to identify patterns and abnormalities that may not be easily visible to human observers, thus potentially increasing the accuracy of forensic assessments (Yang et al., 2020; Camacho &Wang, 2021). For example, deep learning techniques have been used to detect and classify various image manipulations, which can be particularly useful in cases involving digital evidence. This intersection of technology and forensic science is likely to continue evolving, offering new tools and methodologies for forensic anthropologists. Challenges in interpreting imaging data should also be acknowledged. The complexity of human anatomy and the variability in individual cases can make the analysis of imaging findings difficult. Therefore, forensic anthropologists must be cautious in their interpretations, considering the broader context of the case and the limitations of the imaging techniques used (Chen, 2017; Bolliger & Thali, 2015). Continuous education and training in the latest imaging technologies and methodologies are essential for forensic experts to maintain their expertise and make accurate assessments.

The Application of Machine Learning and Deep Learning Algorithms in Forensic Anthropology

1. Machine Learning Applications

Machine learning is a component of AI that can acquire knowledge from data and utilize this knowledge to forecast outcomes. It is categorized into supervised, unsupervised, and semi-supervised learning, each with distinct data structures and applications (Ongsulee, 2017).

1.1. Supervised learning

In supervised learning, the model learns from labeled data to create a link between input data and their corresponding outputs. Through this training process, the model acquires the capability to predict outcomes for new data based on its learning from the provided data. This type of learning is commonly employed for solving classification and regression problems. While classification allows data to be divided into specific categories, regression aims to predict a continuous value. This type of learning is widely used in many fields, such as health, finance, and marketing (Hlad et al., 2021).

Machine learning algorithms have become increasingly common in forensic anthropology, especially in analysing skeletal remains. One of the main applications of machine learning in this field is the determination of biological sex from skeletal features. Classification techniques in machine learning have significantly enhanced forensic anthropologists' ability to accurately determine biological profiles, particularly in estimating sex and age from skeletal remains. Supervised machine learning classification methods applied in forensic anthropology have yielded the following results:

Binary and Multinomial Logistic Regression (BLR and MLR)

Logistic regression is a statistical technique used to examine the relationship between a binary outcome variable and one or more independent variables. In forensic anthropology, binary logistic regression is mainly used to estimate sex, where the outcome variable is binary (male or female), while multiple logistic regression is used for estimating age when considering multiple age categories. The flexibility of logistic regression allows researchers to include various skeletal measurements as predictors, increasing the accuracy of their estimates. Malatong et al., in their study using deep learning techniques, employed a morphometric approach to analyze lumbar vertebrae and demonstrated the effectiveness of logistic regression in determining sex from skeletal remains *(Table 1.)* (Malatong et al., 2022). The study emphasized the importance of using precise measurements and statistical modeling to improve the accuracy of sex estimations.

Table 1. Sex determination accuracies obtained in the study (Comparison of the measurement made while preparing the datasets and the prediction made by the deep learning model) (Malatong et al., 2022).

Endplate Type	Sex	Accuracy (%)
Superior endplate	Female	92.5
Superior endplate	Male	92.5
Inferior endplate	Female	88.5
Inferior endplate	Male	88.5
Superior and inferior endplate	Female	91.0
Superior and inferior endplate	Male	91.0

Kurniawan's research offers a comprehensive analysis of artificial intelligence applications in dental age estimation, focusing on integrating machine learning models with logistic regression techniques to enhance prediction accuracy (Kurniawan, 2024).

Despite the progress made in sex and age estimation using logistic regression, some challenges persist. One significant issue is the variability in skeletal morphology across different populations, which affects the generalizability of models developed from specific datasets. Researchers are increasingly focusing on developing population-specific models that account for morphological differences. Additionally, advanced imaging techniques such as cone-beam computed tomography (CBCT) and 3D modeling are expected to improve the precision of measurements used in logistic regression analyses (Dédouit et al., 2014).

Linear Discriminant Analysis (LDA)

LDA is a statistical technique used to determine the most effective linear combination of features that enhances the distinction between several object categories. In forensic anthropology, Linear Discriminant Analysis (LDA) is especially advantageous for sex estimation when the dependent variable is categorical (male or female), and the independent variables are continuous measures obtained from skeletal characteristics. The method

assumes that predictors are normally distributed and that classes share a common covariance matrix, making it suitable for analyzing anthropometric data (Liebenberg et al., 2015; Bertsatos & Athanasopoulou, 2019).

Bidmos et al. conducted a study on sex estimation using 100 patellae in South Africa. They identified significant differences between male and female measurements using six different measurement parameters and applied stepwise and direct discriminant function analyses to model these differences. The study also employed classical machine learning techniques and feature ranking methods to determine the optimal feature combinations. As a result, the stacking machine learning technique achieved a 90.8% accuracy rate in sex estimation. These findings are consistent with similar studies conducted in other countries, demonstrating the achievement of high accuracy rates *(Table 2.)* (Bidmos et al., 2023).

Table 2. Comparing cross-validated performance of various classifiers and stacked models (Bidmos et al., 2023).

Classification	**Method**	**Accuracy (%)**
Age Classification	NB	76
Sex Classification	NB	77.5

Naïve Bayes Classification (NB)

NB is a classification method that uses Bayes' theorem and assumes that the presence of a specific feature in a class is not affected by the presence of any other feature. This "naive" assumption simplifies the calculation of probabilities, making NB particularly efficient for classification tasks. In forensic anthropology, NB is used to classify skeletal remains into categories such as male or female (for sex estimation) and various age groups (for age estimation) based on skeletal measurements and morphological features. In their study, Hemalatha et

al. used NB to estimate sex and age from dental radiographs, achieving an accuracy rate of 77.5% for sex determination and 76% for age estimation *(Table 3.)* (Hemalatha et al., 2023).

Table 3. Performance comparison (Hemalatha et al., 2023).

Classifier	**Mean**	**Standard Deviation**
LDA	83.85	± 4.47
RF	89.23	3.77
LR	83.85	4.47
K neighbors classifier	83.08	4.56

The variability in skeletal morphology across populations can negatively affect the generalizability of this method, as with other classification methods. As emphasized in the study by Winburn & Algee-Hewitt, it is important to develop population-specific models that account for morphological differences to increase prediction accuracy (Winburn & Algee-Hewitt, 2021).

Decision Trees (DT)

DT belong to the category of supervised learning algorithms that create decision models based on data attributes. Their operational mechanism involves iteratively dividing the dataset into smaller subsets according to the values of input attributes. This process results in a tree-structured model where internal nodes represent attribute-based decisions, branches signify the outcomes of these decisions, and leaf nodes denote class labels. For instance, in the context of forensic anthropology, these labels could be 'male' or 'female' for sex estimation or specific age ranges for age estimation tasks. The simplicity and visual structure of DT make them particularly attractive for forensic applications where interpretability is crucial (Brown et al., 2018).

Traditional methods often rely on morphological assessments that can be subjective and variable in accuracy. However, the application of machine learning algorithms, especially DT,

shows promise in improving the reliability of these predictions. DT allows for clear visualization of decision-making processes by classifying data according to feature values, particularly useful in forensic cases where transparency is crucial (Mohammad et al., 2022; Austin & King, 2016). In this context, machine learning algorithms like DT offer an effective method for classifying and predicting data. Particularly in forensic anthropological applications like sex determination, using such algorithms increases the accuracy and reliability of the obtained data (Nasien, 2023; Öner et al., 2021).

The study by Öner et al. aimed to analyze the accuracy of sex determination using the DT method based on patellar morphometry. With the measurements made, the prediction rate was calculated to be 98.2% for male individuals and 98.4% for female individuals. As a result of the study, they achieved sex determination with high accuracy in the patellar DT analysis *(Figure 1.)* (*Öner et al.,* 2021).

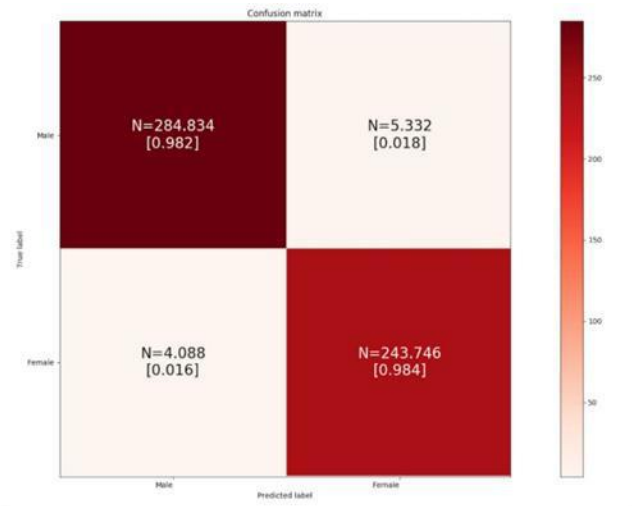

Figure 1. Confusion Matrix for the test set (*Öner et al.*, 2021).

Random Forest (RF)

During its training phase, RF employs a collective learning technique to produce multiple decision trees. For classification tasks, it produces an output based on the most frequent class prediction among individual trees. In regression scenarios, it yields the average of predictions from all trees in the ensemble. This technique is particularly advantageous in processing high-dimensional data and is robust against overfitting, making it suitable

for complex datasets often encountered in forensic anthropology. The RF algorithm works by creating numerous DTs from random subsets of the training data, thus increasing the accuracy and generalizability of the model (Schonlau & Zou, 2020).

Balan et al. evaluated panoramic radiographs using deep learning techniques and compared classification methods. The success percentages of the classification methods used for age estimation in their study are shown in *Table 4.* (Balan et al., 2022).

Table 4. Comparison of classification methods used in age and sex estimation (Balan et al., 2022).

Method	Age Accuracy (%)	Sex Accuracy (%)
RF	91.0	90.0
NB	76.0	77.5
CNN	77.5	78.0
SVM	83.0	82.5
Deep CNN	91.0	91.7
SNCNN	99.6	93.8

In the study conducted by Farhadian et al., data obtained from mastoid process images acquired through CBCT were evaluated to assess differences between sexes. As a result of the evaluation, RF achieved the highest performance percentage at 97% *(Table 5.)* (Farhadian et al., 2020).

Table 5. Evaluation of performance accuracy among various classification models employed in sex determination (Farhadian et al., 2020).

Prediction model	Test Accuracy
SVM	0.927
ANN	0.841
RF	0.969
K-NN	0.909
LR	0.917
NB	0.925
LDA	0.919

Despite the advantages of using RF for sex and age estimation, various challenges persist. One significant issue is the potential for overfitting, where the model becomes too complex and captures noise in the data rather than the underlying pattern. This limitation affects the accuracy of predictions, especially when dealing with small datasets or datasets with high variability (Liebenberg et al., 2024).

Artificial Neural Networks (ANN)

ANN are models inspired by the neural networks found in the human brain. ANN are made up of linked neurons that work together to recognize patterns and anticipate results based on input data. They excel at representing intricate, non-linear connections between input factors and results, which makes them well-suited for tasks like classification and regression.

In forensic anthropology, ANN are used to classify skeletal remains into sex categories and age groups based on various morphological features. In the study by Anic-Milosevic et al., an ANN model was developed using orthodontic measurements of teeth, demonstrating the effectiveness of ANN in sex determination. The study achieved over 80% success rate (Anic-Milosevic et al., 2023).

In another study, Bewes et al. developed a deep-learning ANN model with images obtained from CT scans. The model showed a 95% accuracy rate in sex determination *(Figure 2.)* (Bewes et al., 2019).

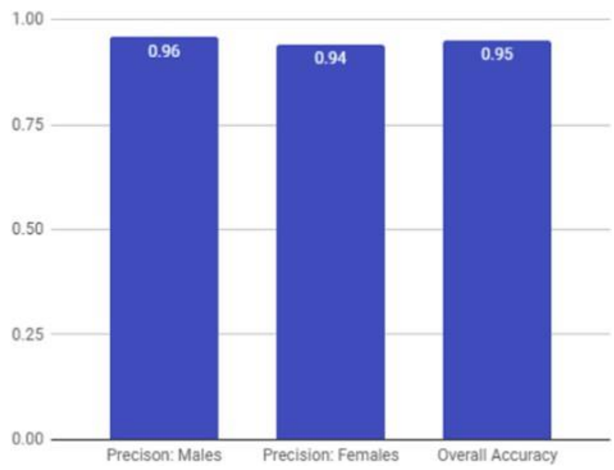

Figure 2. Prediction accuracy percentages of the developed model by sex (Bewes et al., 2019)

Patil et al. examined machine learning and ANN created with panoramic radiographs in their study. The Deep Learning Model created with ANN achieved an 87.2% success rate *(Table 6.)* (Patil et al., 2023).

Table 6. Classification Performance Analysis (Patil et al., 2023).

Class Division	**Model**	**Accuracy**
2-Class	Classification using Deep Learning	87.2

While artificial neural networks (ANN) offer several benefits for estimating sex and age, there are still several challenges to address. One major obstacle is the requirement for extensive, top-notch datasets to efficiently train the models. The effectiveness of ANN is greatly influenced by the quality and quantity of training data, and inadequate data can result in overfitting or underfitting. (Sevim et al., 2016).

Support Vector Machines (SVM)

SVM are used in supervised learning for classifying and analyzing data for

regression. These models work by finding the best hyperplane in a multi-dimensional space to separate different classes of data points. The primary goal is to maximize the margin between classes, and this is calculated based on support vectors, which are the data points closest to the hyperplane. Due to its ability to utilize kernel functions for data transformation into higher dimensions, SVM is especially efficient for classifying data that is not linearly separable (Shan et al., 2022).

Darmawan et al. compared different classification methods to determine sex from finger bone lengths by dividing individuals under 19 years old into age groups. The results of the study are shown in *Table 7.* (Darmawan et. al., 2015).

Table 7. Highest accuracy percentage (%) obtained from three classification techniques for each age group (Darmawan et al., 2015).

Group of age	**SVM Male**	**SVM Female**	**SVM Average**	**ANN Male**	**ANN Female**	**ANN Average**
Newborn–19	63.86	72.46	68.17	69.28	63.47	66.37
16–19	96.67	90.0	93.33	96.67	96.67	96.67
13–15	81.08	68.42	74.67	63.25	69.46	66.37
10–12	70.45	65.85	68.24	65.91	68.29	67.06
7–9	61.11	86.96	75.61	83.33	78.26	80.49
4–6	70.0	21.05	46.15	50.0	52.63	51.28
Newborn–3	76.47	18.75	48.48	64.71	68.75	66.67

Toneva et al. used SVM and ANN to create classification models for sexse prediction based on skull measurements. The results of the evaluation of the measurements obtained from the skull with CT are shown in *Table 8*. The accuracy results for all three methods were over 95%, and the SVM had the highest accuracy at 96.1%. (Toneva et al., 2021).

Table 8. Classification accuracies obtained by SVM, ANN and LR (Toneva et al., 2021).

Algorithm	**Selection**	**Accuracy Males**	**Accuracy Females**
SVM	Full	95.6 ± 1.0	96.5 ± 0.6
	BestFirst	93.7 ± 1.0	96.0 ± 0.7
	GeneticSearch	92.2 ± 1.0	95.8 ± 0.4
ANN	Full	94.2 ± 1.2	95.9 ± 0.7
	BestFirst	91.7 ± 1.3	95.4 ± 0.7
	GeneticSearch	91.9 ± 1.2	94.5 ± 1.2
LR	Full	93.5 ± 0.7	93.5 ± 0.7
	BestFirst	94.7 ± 0.7	95.7 ± 0.6
	GeneticSearch	92.2 ± 1.2	95.7 ± 0.5

1.2. Semi-Supervised Learning

Semi-Supervised Learning is a type of learning where both labelled and unlabelled data are used together. In this type, the model is trained with a limited number of labelled data while improving the learning process by using more unlabelled data. Semi-supervised learning is generally preferred when labelled data is difficult or costly to obtain. This type of learning stands out as an effective method to improve the overall performance of the model, especially in areas with large data sets (Mousavi, 2022). Semi-supervised approaches are either transductive methods, which aim to assign labels to a collection of unlabelled images, or inductive methods, which attempt to develop a classifier that can predict labels for any image in the input space (Jesper et al., 2020). Semi-supervised learning mainly includes semi-supervised classification, semi-supervised clustering, semi-supervised regression, and semi-supervised dimensionality reduction.

1.3. Unsupervised Learning

Unsupervised Learning is a method in machine learning that discovers hidden structures, patterns, or groupings in data by operating on unlabeled data. Unsupervised learning techniques can be listed as K-Means Clustering, Hierarchical Clustering, DBSCAN, Fuzzy C-Means Clustering, Principal Component Analysis (PCA), t-Distributed Stochastic Neighbour

Embedding (t-SNE) (Naeem et. al., 2023).

K-Means clustering divides the data points into K number of predetermined clusters and determines the centre of each cluster. In forensic anthropology, this method can be used to analyze the morphometric properties of bones. For example, morphometric data of bones such as the femur and pelvis can be grouped with the K-Means algorithm, and sex estimation studies can be performed. Hierarchical Clustering creates a dendrogram, a tree-like structure, by combining or dividing the data step by step. This structure visually presents the relationships between the data. In forensic anthropology, Hierarchical Clustering can be useful in comparing the bone structures of different individuals. DBSCAN groups data points according to their density and distinguishes low-density regions as noise. In forensic anthropology, this method can be used to make age estimates by analyzing the density distributions of bones. Fuzzy C-Means Clustering allows each data point to belong to more than one cluster. This flexibility can be useful in forensic anthropology to manage better uncertainties in the age and sex estimates of individuals. Principal Component Analysis (PCA) extracts the most important features of high dimensional data by reducing its size. In forensic anthropology, PCA can help to obtain more meaningful results by reducing the size of the data set when analyzing the morphometric characteristics of bones. For example, age and sex estimates can be made by analyzing the size and shape characteristics of bones with PCA. Autoencoders compress the input data through a series of hidden layers and then use this compressed representation to produce outputs as similar as possible to the original data. This method can be used in forensic anthropology to minimize data loss when analyzing the morphometric properties of bones. t-SNE is a technique that helps visualize complex data in a lower-dimensional space, ensuring that similar data points are clustered together and dissimilar points are spread apart. This method can be useful for visual analyses in forensic anthropology, making the morphometric properties of bones more understandable, and for age and sex

estimation (de la paz-Marin et al., 2015).

1.4. Transfer Learning

Transfer Learning is a technique that enables a model to use the knowledge learnt in one task in another task. For example, features learnt by a model in one dataset can be applied in a similar dataset. This is particularly useful when working with limited data sets. Transfer learning is often used in deep learning and enables pre-trained models to be reused for new tasks. This method allows the model to learn quickly and effectively and use the previously learnt information in the new task.

Ataş conducted a study comparing deep transfer learning architectures and concluded that the DenseNet121 model, using deep transfer learning and a fully automatic technique, could be employed to analyze panoramic dental X-ray images, especially for sex estimation research in forensic sciences *(Table 9.)* (Ataş, 2022).

Table 9. Deep Transfer Learning Models (Ataş, 2022).

Model	**Accuracy**
VGG16	0.8220
ResNet50	0.9260
EfficientNetB6	0.9400
DenseNet121	0.9725

2. Deep Learning Applications

Deep learning, a branch of machine learning, has become a revolutionary technology that employs multi-layer neural networks to understand intricate data representations. This method enables the model to automatically extract features from raw data, allowing it to identify complex patterns and relationships that traditional machine learning techniques may not be able to detect. Deep learning models typically comprise multiple layers, with each layer progressively transforming the input data into higher-level abstractions. The development of network architectures in artificial intelligence has significantly impacted various fields, including forensic anthropology, especially in the study of age and sex estimation. Starting with

classical architectures such as LeNet-5, AlexNet and VGG16, studies have evolved with more advanced models such as Inception, ResNet, ResNeXt and DenseNet. Each of these architectures has unique features that can be used to analyze skeletal remains, which is very important in forensic anthropology (Mohamed et al., 2023). The taxonomic distribution of DCNN architectures is as shown in *Figure 3.* (Khan et al., 2020).

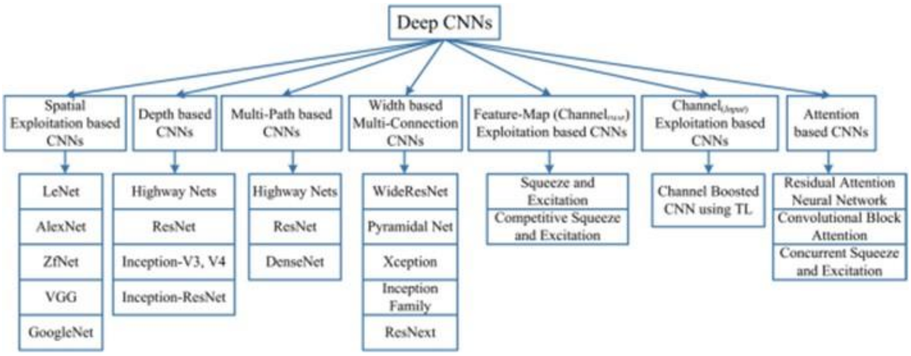

Figure 3. Developmental distribution tree of CNN architectures (Khan et al., 2020).

Deep learning relies on the concept of deriving representations from data without requiring manual feature engineering. In image processing, for instance, the initial layers of a CNN can recognize simple features like edges and textures, while subsequent layers can combine these features to identify more intricate structures such as shapes and objects (Najafabadi et al., 2015). In a study conducted by creating CNN models, Cao et al. performed sex determination from images of coxae bones obtained by CT (Table 10.) (Cao et al., 2022).

Table 10. Performance of CNN models in independent sex prediction

Pelvic region	**Accuracy**
Ventral pubis	0.979 - 1.000
Dorsal pubis	0.959 - 1.000
Greater sciatic notch	0.881 - 0.963
Pelvic inlet	0.926 - 0.987
Ischium	0.800 - 0.909
Acetabulum	0.666 - 0.802

The automatic evaluation of radiographs constitutes a practical application for bone age determination and an ideal target for deep learning. Age estimation based on comparing one or several radiographic images with a reference standard has been used for several decades. Şenel et al. evaluated X-ray films of wrist bones with different deep-learning architecture methods and compared the performance of classifiers (Table 11.) (Şenel et al., 2021).

Table 11. Performance comparisons of classifiers (Şenel et al., 2021).

Classifier	**Training-Testing Data Splitting**	**Metrics**	**Mean**
GoogleNET	70%-30%	MSE	0.0072
		MAE	0.0107
		Acc	0.9137
	80%-20%	MSE	0.0046
		MAE	0.0077
		Acc	0.9406
AlexNET	70%-30%	MSE	0.0148
		MAE	0.0204

		Acc	0.8199
	80%-20%	MSE	0.0090
		MAE	0.0128
		Acc	0.8942
Vgg19	70%-30%	MSE	0.0235
		MAE	0.0300
		Acc	0.7263
	80%-20%	MSE	0.0125
		MAE	0.0163
		Acc	0.9281

Conclusion

The role of AI in forensic anthropology is increasingly recognized in the context of Disaster Victim Identification. Forensic anthropologists play a crucial role in mass disasters where rapid identification of victims is required. The integration of AI technologies automates the comparison of skeletal remains with antemortem records, accelerating the identification process and making the resolution of time-intensive cases more practical (Piraianu, 2023). This capability is especially important in scenarios such as natural disasters or terrorist attacks, as timely identification allows forensic processes to be concluded both quickly and accurately, ensuring justice and preventing potential victimization of the victim's relatives.

Despite the promising developments in AI and machine learning applications in forensic anthropology, challenges remain. Since the effectiveness of machine learning algorithms is highly dependent on the quality and quantity of data used for training, the demand for high-quality training datasets increases as multiple fields of study continue to diversify. In addition, ethical considerations surrounding the use of AI in forensic cases are discussed, particularly in the context of bias issues and the implications of automated decision-making on the legal landscape (Ahmed, 2023). The potential for algorithmic bias raises concerns about the fairness and reliability of AI-assisted forensic analyses. Continuous research and development are required to ensure these technologies are used responsibly and ethically.

Furthermore, integrating AI into forensic anthropology raises questions about the role of human expertise in the analytical process. While AI can improve the efficiency and accuracy of forensic investigations, it is important to remember that human bias and experience remain critical components of forensic analysis. Collaboration between forensic anthropologists and AI technologies can enable algorithms to capture contexts and structures that human experts cannot fully capture with classical methods, leading to more robust and reliable results (Mladenović et al., 2023). This collaborative approach will help reduce the risks associated with over-reliance on automated systems, performing forensic analyses with the highest scientific rigour *Figure 4*.

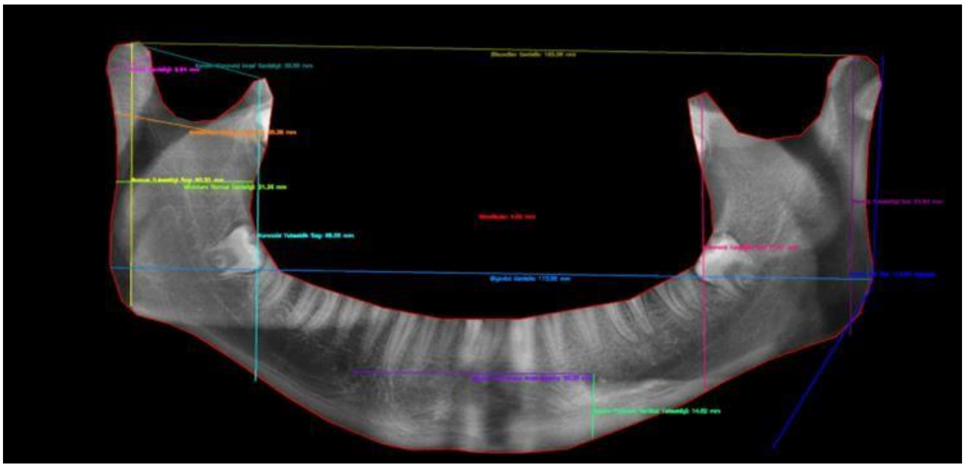

Figure 4. Machine learning training material for the mandibular identification model, which will enable sex determination and stone prediction through the collaboration of machine learning and panoramic radiography in our ongoing work.

Human-AI collaborations in forensic identification studies, conducted after events such as wars and mass murders—regardless of the passage of time—in both murder cases and mass disasters, will provide significant benefits to humanity and society as research advances and developments progress. Such collaborations will help prevent law and human rights violations under all circumstances and enable law enforcement officers, crime scene investigators, forensic scientists, and lawyers involved in the judicial process to work in much healthier conditions.

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SIMULTANEOUS ANALYSIS OF ORGANIC AND INORGANIC GUNSHOT RESIDUE

Harun ŞENER, Hatice SOYTÜRK

Chapter 3

Simultaneous Analysis Of Organic And Inorganic Gunshot Residue

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Introduction

Gunshot Residue (GSR) constitutes a significant type of trace evidence essential for investigating firearm-related offenses. GSR consists of a mixture of particles originating from both burned and unburned primer, propellant gases, bullet components, and material expelled from the firearm itself during the discharge (Basu, 1982; Carneiro et al., 2023; Wolten, n.d.; Lucas et al., 2019). These particles, due to their minuscule size, can transfer to the shooter's hands, clothing, face, and hair. They can also deposit on surrounding surfaces, including those of victims and the environment near the discharge site (Lucas et al., 2019). Factors that affect the quantity of gunshot residue (GSR) and its dispersal range encompass the type of firearm, the movement of the weapon, the type of ammunition utilized, the number of shots discharged, and the existing environmental conditions (Şener, 2020).

In forensic investigations, detecting GSR on individuals or objects can aid in linking residues to a specific firearm incident (Rosengarten et al., 2021). Establishing a relationship between a suspect and actions connected to a shooting event is crucial in cases involving firearms. GSR collected from suspects, victims, or the crime scene provides valuable insights. However, because samples are often collected at varying times post-incident, forensic scientists must consider factors such as GSR transfer and persistence during evidence interpretation. Additionally, understanding the formation of GSR and its presence in the ambient environment is essential. Nonetheless, significant challenges remain in identifying and interpreting GSR, presenting ongoing difficulties for the forensic science community (Şener, 2020).

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GSR refers to inorganic gunshot residues (IGSR) and organic gunshot residues (OGSR). The current analytical method for detecting IGSR relies on identifying particles of specific size and morphology that contain various combinations of elements, including barium, antimony, lead, gadolinium, titanium etc. (Dalby et al., 2010; Saverio Romolo & Margot, 2001). The residues originate from the primary source, and the analysis is performed using scanning electron microscopy (SEM) with X-ray spectroscopy. In recent years, OGSR analyses have been conducted alongside heavy metal analyses to evaluate ammunition designed to be safe for both the shooter and the environment, as it is devoid of heavy metals. International standards have not yet validated a standardized analysis method for OGSR analyses (Şener, 2021).

Forensic scientists must have a solid understanding of GSR traces, including their transfer, persistence, and prevalence, to develop reliable methodologies for detecting, collecting, analyzing, and interpreting these residues in forensic investigations. This chapter seeks to summarize the current knowledge regarding GSR, focusing on residues transferred to individuals or objects not directly involved with the shooter, such as bystanders or surrounding surfaces. Additionally, it outlines the existing methods for GSR detection and analysis, evaluates the strengths and limitations of the mentioned approaches. Also, it provides recommendations for future research to advance forensic GSR studies.

1. GSR Production and Composition

Gunshot residues (GSR) are released from firearms in the form of inorganic particles and organic vapors (Blakey et al., 2018; Vander Pyl, Feeney, et al., 2023). The two main types of GSR are distinguished for methodological purposes, as different methods are typically utilized for their detection and analysis. IGSR originates mainly from the firearm's capsule and several metallic elements, including the barrel, bullet, and cartridge case, while OGSR is a product of the incomplete combustion of the propellant. After a shooting incident, projectile residue accumulates on surfaces near the discharged firearm, including the shooter's hands and face, as well as on

the intended targets (Vander Pyl, Feeney, et al., 2023).

1.1. Inorganic GSR

In forensic science, Inorganic Gunshot Residue (IGSR) consists mainly of metallic particles derived from the primer, which is a mixture of fuel, oxidizer, and explosive that reacts during firearm discharge. The particles may originate from the bullet core, cartridge casing, and the mechanical wear of the firearm's moving components (Szakas & Gundlach-Graham, 2024). The primer's explosive reaction comprises three essential components: lead styphnate ($C_6HN_3O_8Pb$) as the primary explosive, barium nitrate ($Ba(NO_3)_2$) functioning as an oxidizer, and antimony trisulfide (Sb_2S_3) as a fuel (Feeney et al., 2020; Vander Pyl, Feeney, et al., 2023). Forensic laboratories commonly employ Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM/EDX) to analyze IGSR. This technique demonstrates high sensitivity and specificity, enabling forensic scientists to identify inorganic particles within a sample and ascertain their elemental composition and morphology with remarkable precision (White, 1987; Redouté Minzière et al., 2023).

While conventional sinoxid-type primers, which release toxic elements like lead, barium, and antimony, remain in widespread use, there is a growing shift toward heavy metal-free primers (Figure 1). These environmentally friendly primers incorporate elements like sulfur (S), aluminum (Al), potassium (K), titanium (Ti), gadolinium (Gd), gallium (Ga), silicon (Si), and zinc (Zn) (Charpentier & Desrochers, 2000; Gunaratnam & Himberg, 1994; Romanò et al., 2020). This transition reflects increased awareness of environmental and health concerns related to releasing toxic substances from traditional ammunition.

1.2. Organic GSR

Organic gunshot residue (OGSR) mostly originate from the smokeless powder and primer contained in the cartridge casing. Smokeless powders consist of intricate combinations of explosive materials and various additives, including stabilizers, plasticizers, flash inhibitors, coolants, deterrents, surface lubricants, dyes, and other agents that enhance performance (Vander Pyl, Feeney, et

al., 2023). Upon the discharge of a firearm, incomplete combustion, evaporation, and condensation processes result in the presence of unburned smokeless powder constituents and their degradation products in gunshot residue (Minzière et al., 2023). The unburned particles possess significant forensic value, offering insights into the type of ammunition utilized and the circumstances surrounding the discharge.

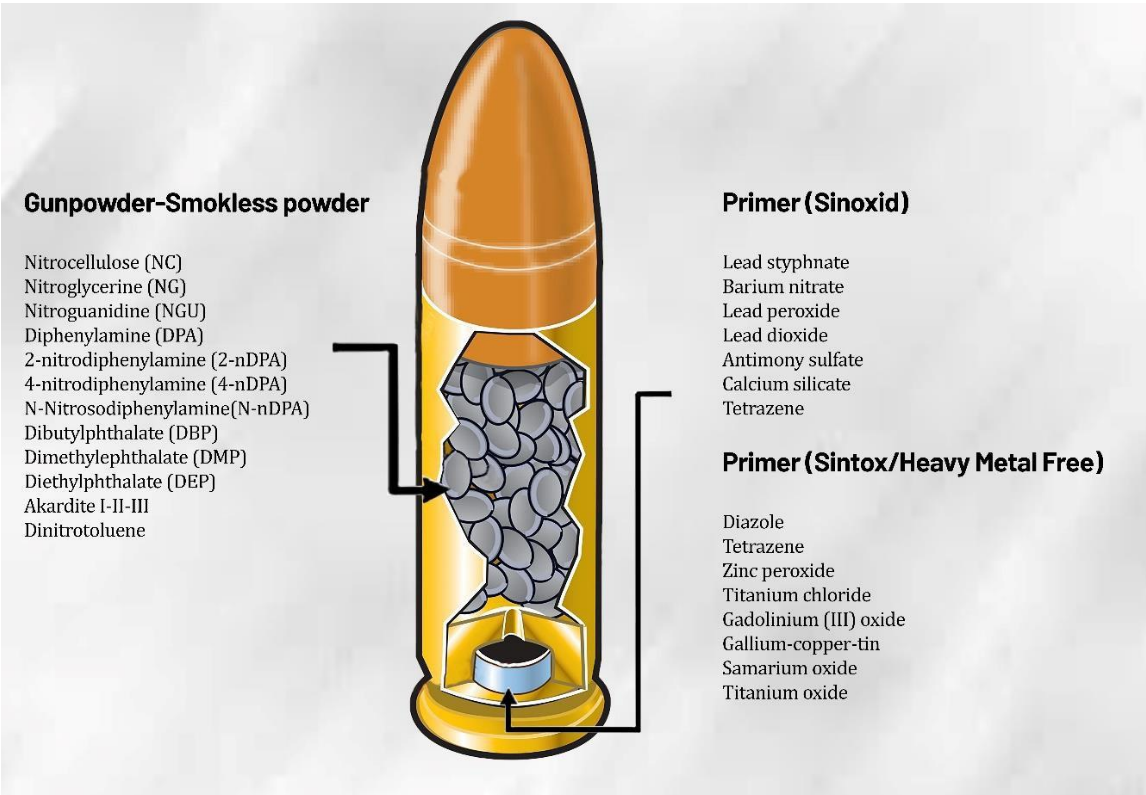

Figure 1. Composition of firearm ammunition

The principal propellants in firearms are smokeless powders, categorized into three types according to their chemical composition:

- Single-base powders: Consisting exclusively of nitrocellulose (NC) as the singular explosive element.
- Double-base powders comprise both nitrocellulose (NC) and nitroglycerin (NG).
- Triple-base powders comprise nitrocellulose (NC), nitroglycerin (NG), and nitroguanidine (NQ) (Taudte et al., 2015).

Notable volatile organic compounds used as additives in smokeless powders

include stabilizers such as diphenylamine (DPA) and its isomers; 2-nitrodiphenylamine (2-nDPA), 4-nitrodiphenylamine (4-nDPA), and N-nitrosodiphenylamine (N-nDPA) along with methyl centralite (MC) and ethyl centralite (EC) (Figure 1) (Taudte et al., 2015). These compounds are critical for extending the shelf life of smokeless powder and minimizing the risk of accidental ignition, thereby enhancing the stability and safety of ammunition.

Forensic scientists concentrate on elucidating the composition of organic residues to enhance methodologies for detecting and analyzing gunshot residue (GSR). Examining organic gunshot residue enables forensic specialists to identify the type of ammunition employed, assess the firing distance, and possibly connect a suspect to a shooting incident. This analytical methodology is essential in criminal investigations pertaining to firearms.

2. The Evidence of Gunshot Residue

2.1- Transfer

Gunshot residue (GSR) can be transferred through various mechanisms. Primary transfer occurs directly after a firearm is discharged, leading to the deposition of GSR on the shooter's hands, which can then spread to other body parts or clothing. Secondary transfer happens when a clean surface comes into contact with a surface already contaminated with GSR, such as handling a fired weapon, shaking hands with a shooter, or touching other contaminated surfaces. Tertiary transfer involves further contamination, where a surface affected by secondary transfer contacts another, continuing the spread of GSR. The behavior of GSR particles is influenced by their physicochemical properties, including how they interact with surfaces like human skin (Blakey et al., 2018; Vander Pyl, Dalzell, et al., 2023).

The factors influencing GSR transfer encompass the elapsed time since the shooting, the specific firearm and ammunition utilized, the distance to the target, and subsequent activities following the shooting. Research demonstrates that organic gunshot residue (OGSR) is frequently concentrated on the hands and forearms owing to its volatile and lipophilic properties, facilitating absorption into the the skin epidermal layer. As a result, OGSR exhibits

reduced vulnerability to secondary transfer (Demircioğlu et al., 2024; Feeney et al., 2020, 2022; Hofstetter et al., 2017; Moran, 2013). In contrast, inorganic gunshot residue (IGSR) particles, which adhere to the external surfaces of skin and clothing, are more susceptible to secondary and tertiary transfers when exposed to physical disturbances (Blakey et al., 2018; Feeney et al., 2020, 2022; French et al., 2014). If the shooter is not identified and samples are not collected promptly after the discharge, the increased possibility of secondary transfer and the depletion of GSR particles poses a significant challenge for the investigation (Demircioğlu et al., 2024).

Research shows that OGSR is not easily transferred through casual contact but can be lost during activities that involve prolonged or intense contact, such as rubbing hands or washing with soap (Vander Pyl, Dalzell, et al., 2023). Furthermore, the dominant hand, which typically accumulates more GSR, is also more vulnerable to transfer than the non-dominant hand (Maitre, Horder, et al., 2018; Maitre, Kirkbride, et al., 2018).

An important consideration in GSR analysis is the memory effect—the potential impact of the firearm's shooting history on collected samples. The type of ammunition last fired, whether it produces OGSR or IGSR, can influence the results, particularly in the initial shots after changing ammunition types, though this effect gradually diminishes over time (Donghi et al., 2024; MacCrehan et al., 2001). Interpreting GSR analyses requires caution because the exact type of ammunition used is unknown, and prior ammunition can significantly influence the findings.

2.2. Persistence (t > 0)

Gunshot residue (GSR) does not persist indefinitely on the shooter's hands or other surfaces, gradually diminishing over time. Criminals may take deliberate actions such as washing hands, changing clothes, showering, or wearing gloves to remove or prevent GSR contamination. Therefore, it is imperative to obtain samples from suspects promptly following a shooting and to implement necessary measures to prevent the destruction of potential evidence (Arndt et al., 2012; Maitre, Horder, et al., 2018).

Comprehending the persistence of GSR is essential for enhancing the detection and analysis of OGSR. This knowledge aids various stakeholders engaged in forensic investigations. Forensic investigators and laboratories offer critical insights into the probability of obtaining positive gunshot residue (GSR) results, particularly when there is a temporal gap between the shooting incident and sample acquisition. It assists forensic specialists in analyzing OGSR outcomes in situations requiring differentiation between individuals who discharged a firearm and those uninvolved, as well as in evaluating the participation of individuals in a firearm-related event (Maitre, Horder, et al., 2018).

Most research indicates a rapid decline in GSR levels within the first few hours following a shooting (Gassner & Weyermann, 2016; Maitre, Horder, et al., 2018; Minzière et al., 2022). In 1975, it was shown that neutron activation analysis (NAA) could detect barium (Ba) and antimony (Sb) residues on shooters' hands for up to six hours post-discharge (JW, 1995; Romanò et al., 2020). Another study by Chávez Reyes et al. observed a decrease in GSR levels within four hours of the shooting using nasal stubs as a collection method (Chávez Reyes et al., 2018). Maitre, Horder, and colleagues, as well as Gassner & Weyermann, reported a significant decrease in OGSR levels within the first-hour post-discharge, though they were still detectable up to four hours later through liquid chromatography-mass spectrometry (LC-MS/MS) analyses. They also noted that handwashing could lead to partial or complete removal of OGSR, with the lipophilic nature of certain OGSR compounds playing a key role in their persistence on the skin (Bonnar, 2023; Gassner & Weyermann, 2016; Maitre, Horder, et al., 2018). Additionally, Rosengarten et al. found that GSR could be detected on towels, even if suspects attempted to wash off residues by showering (Rosengarten et al., 2021).

The findings suggest that the behavior of OGSR depends heavily on the physicochemical properties of the compounds, while the behavior of inorganic GSR (IGSR) is more influenced by physical properties. IGSR particles tend to persist unless mechanically disturbed, whereas OGSR is more easily diminished through daily activities and can be more readily

transferred (Vander Pyl, Dalzell, et al., 2023).

2.3. Prevalence

Prevalence refers to the frequency with which gunshot residue (GSR) is detected in a specific population or environment, including cases where GSR is found on individuals not directly involved in firearm-related incidents. This can occur due to secondary contamination, such as through contact with police officers or other individuals who may have handled firearms or GSR-contaminated surfaces during custody or investigation procedures (Minzière et al., 2022). Understanding the levels of GSR prevalence among law enforcement personnel is crucial. It enables forensic experts and police departments to adjust their operational procedures to find ways to minimize the risk of contamination during arrests (Cook, 2016; Stamouli et al., 2021). Studies analyzing IGSR among civilian populations in various countries have generally detected a low number of characteristic particles (Minzière et al., 2022).

3. GSR Collection and Analysis

3.1. Collection

Selecting the most suitable method for collecting gunshot residue (GSR) from various surfaces is essential to maximize the efficiency of collection and ensure reliable forensic analysis (Minzière et al., 2023; Shrivastava et al., 2021). Common tools for collecting GSR include alcohol wipes, adhesive tapes, stubs, and cotton swabs. The optimal method for collecting gunshot residue (GSR) depends on both the type of surface being sampled and the analytical technique that will be employed for residue detection. A well-chosen collection method should be simple to use, quick, and accurate, while also being portable and adaptable for crime scene use (Şener, 2021).

When gathering gunshot residue evidence, it's vital to follow specific protocols to maintain the integrity of the samples:

- **Personal Hygiene and Glove Use:** The forensic specialist should thoroughly wash their hands before starting the collection process. It's crucial to change gloves between handling

each of the suspect's hands to prevent cross-contamination.

- **Preventing Evidence Loss:** Ensure the suspect does not wash their hands or touch other surfaces. Such actions can result in the loss or contamination of GSR evidence.
- **Documenting Dominant Hand and Sample Collection:** Record whether the suspect is right-handed or left-handed in the official report. Begin swabbing from the palm of the dominant hand, as it's more likely to contain significant residue.
- **Preserving Protective Bags:** If the suspect's hands were secured in protective bags to prevent contamination, do not discard these bags. Place them in separate, labeled containers for further examination.

3.2. Analysis

Gunshot residue analysis can help us link the suspect to the incident, show the origin of the incident (homicide, suicide, etc.), determine the distance and direction of fire, and confirm the veracity of a statement (Hofstetter et al., 2017). Existing analysis methods for GSR mainly focus on the analysis of inorganic GSR consisting of metallic particles from primers, bullets, cartridges, and weapons.

3.2.1. IGSR Analysis

In the past, dermal nitrite testing, paraffin testing, and Harrisson and Gilroy Method Neutron Activation Analysis (NAA) were used. Today, Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), Electron Scanning Energy Dispersive X-ray Spectrometry (SEM-EDX), and atomic absorption spectrometry (AAS) are used (Meng H.H., 1997; Krishnan, Gillespie, 1971).

Today, Inorganic Gunshot Residue (IGSR) is analyzed using Scanning Electron Microscopy with Energy Dispersive X-ray Spectrometry (SEM/EDX) following the ASTM E1588-20 standard guidelines. This method involves evaluating GSR and GSR-like particles from environmental sources based on their morphology and elemental composition. The chemical composition is categorized into GSR characteristic particles, which include lead (Pb), barium (Ba), and antimony

(Sb); particles consistent with GSR, such as PbBa or BaSb; particles commonly associated with GSR, such as Ba, Pb, or Sb; and combinations like GdTiZn or GaCuSn found in heavy metal free ammunitions. This highly sensitive and specific technique allows for the detection of inorganic particles within a sample, providing detailed information regarding their elemental composition and morphology (R.S White, 1987; Redouté Minzière et al., 2023).

The SEM-EDX method has two advantages:

1- It allows the shot residue to retain its particle structure and allows the particle morphology to be photographed.
2- All elements emit x-ray radiation at characteristic wavelengths when excited by incident electrons. This makes it possible to record the chemical composition of each particle (Bonnar, 2023).

However, the time required for the characterization of a single sample via SEM-EDX, combined with the increased use of heavy metal-free ammunition that contains elements commonly found in the environment, poses challenges. Additionally, the transfer and persistence of IGSR particles complicate the interpretation of results. To address these limitations and improve the efficiency and reliability of GSR evidence, research on OGSR analyses is expanding. OGSR analyses aim to overcome the complexities associated with IGSR by focusing on organic compounds, potentially providing more robust and specific forensic insights.

3.2.2. OGSR Analysis

Raman Spectrometry, Fourier Transform Infrared Spectrometry (FTIR), Ion Mobility Spectroscopy (IMS), Gas Chromatography-Mass Spectrometry (GC-MS), Liquid Chromatography-Mass Spectrometry (LC-MS), Infrared Spectroscopy (IR), and Capillary Electrophoresis (CE) are commonly used to identify organic gunshot residues (OGSR). However, an internationally recognized standard method for OGSR analysis has not yet been established. There are, however, standard practice recommendations for the analysis of OGSR using LC-MS or GC-MS (OSAC, 2022a, 2022b).

Recent studies show that OGSR offers supplementary insights in examining shooting incidents, particularly with ammunition lacking heavy metals (Feeney et al., 2020; Minzière et al., 2022; Redouté Minzière et al., 2023). OGSR has also been studied to contribute to estimating the distance or time of a shot, but there is no standardized method, such as the sodium-rhodizonate test, based on the lead presence (López-López & García-Ruiz, 2014; Redouté Minzière et al., 2023; Zeichner, 2003).

Compounds like methyl centralite (MC) and ethyl centralite (EC) are primarily used in the production of smokeless gunpowder, making them some of the most distinctive constituents of firearm shot residues. Diphenylamine (DPA) has various industrial applications; however, it is considered a defining feature of gunshot residue (GSR) when detected alongside its nitrated derivatives like N-nitrosodiphenylamine (N-nDPA), 2-nitrodiphenylamine (2-nDPA), or 4-nitrodiphenylamine (4-nDPA) (Maitre, Horder, et al., 2018).

Conclusion

Gunshot residue (GSR) analysis provides crucial information that can link a suspect to a crime, confirm whether a weapon was fired, identify bullet entry and exit holes, and help determine the cause of death, whether it be homicide, self-defense, or suicide (Krishna & Ahuja, 2024; López-López et al., 2013). However, interpreting GSR results requires careful consideration to avoid wrongful convictions. The primary aim of GSR detection and analysis is to determine whether the detected traces are indeed firearm-related shot residues. If confirmed as shot residue, the level of activity, specifically the transfer level, must be established. It is critical to distinguish between primary transfer (direct firearm discharge) and secondary or higher-level transfers (French et al., 2014; Hofstetter et al., 2017; Minzière et al., 2022; Werner et al., 2020). With the advent of heavy metal-free ammunition, current standard procedures are insufficient for evaluating the transfer and persistence mechanisms of organic gunshot residue (OGSR), particularly when determining firing distance. To address these challenges, research into OGSR analysis has increased, focusing on conducting

simultaneous or sequential analyses of inorganic GSR (IGSR) and OGSR. Data on transfer, persistence, and prevalence are essential for developing interpretation models and incorporating OGSR analysis into routine forensic laboratory practices (Redouté Minzière et al., 2023). It is strongly recommended that IGSR and OGSR analyses be performed together to ensure more comprehensive forensic results (Taudte et al., 2014).

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THE SIGNIFICANCE OF POSTMORTEM IMAGING IN THE EVIDENCE ANALYSIS

Ceren AKAGÜNDÜZ AYRANCIOĞLU

Chapter 4

The Significance of Postmortem Imaging in the Evidence Analysis

CEREN AKAGÜNDÜZ AYRANCIOĞLU[1]

1. Introduction

Postmortem imaging has become a crucial tool in forensic medicine, particularly in the field of forensic pathology. Although conventional autopsies have long been the standard for death investigations, postmortem imaging adds a noninvasive, detailed layer of information that can both complement and, in some cases, substitute traditional methods. Postmortem imaging with technologies such as digital radiography (DR), computed tomography (CT), and magnetic resonance imaging (MRI) offers a visual roadmap of internal anatomical structures and preserves the integrity of the body while providing critical insights. In modern forensic science, the accuracy and precision of evidence analysis are paramount. Postmortem imaging has emerged as a transformative tool in this arena, reshaping how forensic pathologists and medical examiners investigate deaths. The ability to capture detailed, three-dimensional, and reconstructed images of a body using advanced imaging technologies has revolutionized the gathering and analysis of evidence. Additionally, postmortem imaging supports legal proceedings by providing visual evidence and documentation, making it an essential tool in modern forensic pathology. Furthermore, the digital nature of the images allows for repeated review, consultation with experts, and preservation of evidence for future reference, ensuring that no critical details are overlooked (*Clemente, La Tegola, Mattera, Guglielmi, 2017;* Decker et al., 2019; *Elifritz, Nolte, Hattch, Adolphi, Gerranrd, 2014; O'Donnell, Woodford, 2008)*

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This chapter aims to provide essential information on postmortem imaging techniques and overview the existing literature on medical imaging from a forensic viewpoint. This chapter is going to assess the definition, purpose, and importance of postmortem imaging, its applications, and techniques and compare various imaging methods that enhance forensic investigations in detail.

2. Definition, Purpose, and Importance of Postmortem Imaging

Postmortem imaging refers to the use of radiological techniques, such as ultrasonography (US), DR, CT and MRI, to examine a body after death. These technologies allow for detailed tissue analysis without the need for dissection (*Clemente et al., 2017; Decker et al., 2019*; *Elifritz et al., 2014; O'Donnell, Woodford, 2008).*

The primary purpose of postmortem imaging is to provide a noninvasive, accurate, and rapid assessment of the internal structures of the body to assist in determining the cause, origin, and mechanism of death. Unlike traditional autopsies, which require incisions and physical manipulation of the body, postmortem imaging allows medical examiners to view internal structures without intervention. Moreover, it can reveal critical evidence that might be overlooked during conventional autopsy. Beyond forensic applications, postmortem imaging serves as a valuable tool in medical research, offering insights into disease progression, congenital abnormalities, and undiagnosed or underlying medical conditions that may have contributed to the individual's death (*Clemente et al., 2017; Decker et al., 2019*; *Elifritz et al., 2014; O'Donnell, Woodford, 2008).*

The importance of postmortem imaging lies in its ability to revolutionize the way forensic and medical professionals approach death investigations and pathology. Postmortem imaging preserves the integrity of the body, making it particularly valuable in situations where the nature of a case limits the use of standard dissection, such as disintegrated corpses like decomposed or burned bodies. Most postmortem imaging technologies help improve diagnostic accuracy by providing precise, high-resolution, three-dimensional, cross-sectional, and

reconstructed body images, offering detailed insight into the death investigation without the need for invasive procedures. In addition to forensic applications, postmortem imaging plays an essential role in medical research and education. This enables the examination of disease progression in ways that may not have been detectable during life. It will contribute to the advancement of medical knowledge and the development of new diagnostic and treatment strategies. Furthermore, postmortem imaging enhances disaster victim identification efforts by enabling rapid, noninvasive assessment of bodies during mass casualty events, where rapid and efficient identification is paramount. Its ability to store and archive digital data for future analysis also ensures that cases can be reviewed or reanalyzed long after the initial examination between antemortem and postmortem data. Considering that a healthy autopsy can only be performed once after death, postmortem imaging is crucial for the re-evaluation of cases. Overall, postmortem imaging is a crucial tool that bridges forensic science, clinical practice, and medical education, offering greater accuracy and efficiency (*Clemente et al., 2017; Decker et al., 2019*; *Elifritz et al., 2014; O'Donnell, Woodford, 2008).*

3. Applications of Postmortem Imaging

Postmortem imaging is particularly useful in various cases where traditional autopsy methods are limited, impractical, or in need of enhancement. Applications of postmortem imaging in some key scenarios are especially useful, such as the detection of foreign bodies, determination of age, victim identification in mass disasters, examination of disintegrated corpses (decomposed or burnt bodies), pediatric and neonatal deaths, asphyxiation cases, violent deaths, homicides and abuse, accidental deaths, clinical research, and missed diagnoses.

Detection of Foreign Bodies: Postmortem imaging plays a crucial role in detecting foreign bodies within a deceased individual. It offers a noninvasive method to identify the anatomic location, shape, and number of objects that may have contributed to or resulted in the death. Technologies that, in particular, allow X-ray imaging,

such as DR and CT, are particularly effective for visualizing foreign materials or medical devices that may be embedded in the body.

In forensic cases involving gunshot wounds, postmortem imaging can clearly identify the trajectory of a bullet, its exact location, and any internal damage caused along its path, providing critical evidence without the need for an autopsy. Similarly, in cases involving stabbings or explosions, postmortem imaging can reveal fragments of knives or debris, allowing forensic experts to reconstruct the event and analyze the impact of these objects on the body. This is particularly important when dealing with fragmented foreign bodies that may be dispersed throughout various tissues or organs *(Magnin,Grabberr, Michaud., 2020; Woźniak, Moskała, Rzepecka-Woźniak., 2015).*

Postmortem imaging is also beneficial for identifying medical devices like pacemakers, implants, and surgical instruments left inside the body, helping to differentiate accidental causes from intentional harm (*De Angelis et al., 2020).*

In the investigation of smuggling cases, particularly when identifying the presence of illicit materials or contraband hidden in a deceased individual's body. Postmortem imaging technologies are highly effective in detecting foreign objects that may have been ingested, inserted, or surgically implanted during smuggling operations. These objects range from drug packets, capsules, and balloons to high-value items like precious stones, currency, and electronics. Postmortem imaging can identify the exact number, size, condition (ruptured or not), and location of packages inside the gastrointestinal tract, body cavities, or surgically created compartments *(Abedzadeh, Iqbal, Bastaki, Pierre-Jerome, 2019; Hamid, Rashid, Saini, 2012).*

In cases of choking, a blockage in the air passages, often due to food or foreign material, leads to asphyxiation or fatal events mechanisms like asphyxia or stimulation of the autonomic nerve plexus. Advanced postmortem imaging techniques, such as CT and MRI are commonly used to detect obstructions in the trachea, bronchi, and upper

airways. These modalities can visualize the size, location, and type of the obstructing material, offering valuable insight into the case and helping to detect secondary signs of asphyxia, such as pulmonary edema and aspiration, which can further support the diagnosis. These imaging findings complement traditional autopsy techniques, aiding in confirming airway obstruction as the primary cause of death and providing essential information for legal and medical inquiries *(Iino, O'Donnell, 2010; Oesterhelweg, Bolliger, Thali, Ross, 2009).*

Another significant benefit of postmortem imaging in cases of foreign bodies is its ability to preserve digital records for further examination and consultation with experts in forensic medicine, law enforcement, and customs. These images can be used as evidence in court, providing a clear, objective visualization of the items and their impact on the deceased.

Determination of Age: Medical imaging is a powerful tool in the determination of age, both antemortem and postmortem, and it provides forensic experts with noninvasive methods to estimate age based on skeletal and dental analysis. Using X-ray imaging techniques, such as DR and CT, the development and degeneration of bones, dentition, closure of sutures, ossification center development, epiphyseal plate maturation, and the condition of articular surfaces can be assessed to estimate an individual's age *(Barszcz, Woźniak, 2024)*

The degree of bone growth and tooth development are key indicators of age. For example, the presence or absence of certain primary and permanent teeth, the stage of the eruption, root development, tooth wear, and the apposition of secondary dentin can help estimate the age from dental radiographs *(Panchbhai, 2011; Limdiwala, Shah, 2013; Chulamanee, Panyarak, 2023)*

Additionally, epiphyseal plate maturation in long bones is a reliable marker. As the body matures, these plates fuse, and the timing of this fusion can be tracked using imaging to estimate age. (*Khatam-Lashgari, Harying, Villa, Lynnerup, Larsen, 2024; Lopatin, Barszcz, Woźniak, 2023)*

In adults, age-related degenerative changes in bones,

particularly in the spine and joints, and the condition of articular surfaces can help forensic experts estimate age *(Adams, Butler, Fuehr, Olivares-Pérez, Tamayo et al., 2024)*

Changes in the symphysis pubis and sternal rib ends are also commonly used indicators of age. The symphysis pubis undergoes predictable morphological changes throughout life, which can be visualized through imaging and correlate with established age ranges. Similarly, the ribs exhibit progressive ossification, and their degree of calcification and changes in shape over time can serve as additional markers for age determination *(Chiba et al., 2014; Sodhi et al., 2016; Monum, 2019)*

Medical images also allow for the preservation of digital records, facilitating further consultation or comparison with pre-existing medical images. The noninvasive nature of medical imaging also respects the integrity of the body while providing detailed anatomical information necessary for forensic age estimation, making it an essential tool in modern forensic practice.

Victim Identification in Mass Disasters: Postmortem imaging is an indispensable tool in mass disaster scenarios, where rapid, efficient, and accurate identification of victims is essential for humanitarian and legal purposes. In the aftermath of large-scale disasters such as earthquakes, floodings, tsunamis, plane crashes, terrorist attacks, or industrial accidents, the condition of victims' bodies may be severely compromised due to trauma, burns, or decomposition, making traditional visual identification methods unreliable or impossible. Advanced imaging techniques, like CT and MRI, allow forensic teams to examine bodies, even under extreme conditions, non-invasively. In mass disasters, postmortem imaging offers the ability to scan multiple bodies quickly, preserving crucial anatomical details such as dental records, bone structure, and the presence of medical devices like pacemakers, prosthetics, and implants *(De Angelis et al. 2020; Argo et al., 2020; Jackowski, Thali, 2009).*

Dental images like DR, CT and cone beam computed tomography (CBCT) play a critical role in disaster victim identification due to tooth durability. Detailed images of the teeth,

jaws, and palatal rugae reveal the individual dental work, such as fillings, crowns, or root canals, and provide a reliable match with antemortem dental records, making dental images a cornerstone of postmortem identification. *(De Angelis et. al. 2020; Jackowski, Thali, 2009; Viner, Robson; 2017; Forrest, 2019)*

Scans of sinus cavities, particularly DR and CT, are frequently used in forensic identification because their shape, dimensions, and patterns vary from person to person, allowing for comparison with pre-existing medical records to confirm identity *(De Angelis et al., 2020; Ruder, 2012; Deloire, 2019).*

In patients who have undergone orthopedic surgeries or medical implants, postmortem imaging can reveal the presence of medical devices implanted in the body, such as pacemakers, stents, joint replacements, plates, screws, pins, or other prosthetic devices. These medical devices often have serial numbers or other identifying features that can be traced back to medical records, providing another method for identifying individuals, especially in cases in which decomposition has progressed to the point where other identification methods are less effective. *(De Angelis e. al., 2020; Jackowski, Thali, 2009; Sidler, Jackowski, Dirnhofer, Vock, Thali., 2007; Numata, Makinae, Yoshida, Daimon, Murakami., 2017)*

Beyond identification, postmortem imaging assists in documenting trauma and injury patterns, which helps forensic pathologists reconstruct the circumstances of death. This is particularly important when the cause of a disaster is under investigation, such as airplane crashes, terrorist attacks, or industrial accidents *(Kahana, Ravioli, Urroz, Hiss., 1997).*

The ability to quickly process and examine multiple bodies using imaging reduces the need for immediate invasive autopsies, which can be time-consuming and stressful in mass casualty situations. Furthermore, digital data captured through imaging can be stored and reviewed later, which is particularly valuable in large-scale investigations that may take months or years to complete. The digital nature of postmortem imaging also enables

forensic experts to store and share findings with other professionals for second opinions or further analysis, thereby enhancing the overall efficiency of the identification process. Overall, postmortem imaging accelerates victim identification in mass disaster situations and preserves critical forensic evidence, ensuring the investigation is thorough and accurate for legal and humanitarian purposes.

Examination of Disintegrated Corpses (Decomposed or Burnt Bodies): Traditional autopsies can be challenging when bodies are in advanced stages of decomposition or have been subjected to fire. In such cases, x-ray imaging techniques such as DR and CT can reveal bone fractures, dental conditions, medical devices, or the presence of foreign objects that aid in identification and evidence collection.

In cases of advanced decomposition, in which the external tissues have deteriorated to the point of obscuring anatomical landmarks, postmortem imaging can reveal distinguishing anatomical characteristics, such as healed fractures, surgical implants, and dental condition, all of which can be used to match the deceased to antemortem records. Imaging allows forensic experts to visualize bones, remaining soft tissues, and internal organs, which may offer clues to the cause of death or help identify the individual. For instance, CT scans can clearly show skeletal injuries, fractures, or the presence of foreign objects, even when the body is extensively decomposed. Imaging can also visualize gas accumulation and other postmortem changes in tissues and organs, which is common during decomposition, and identify patterns of fluid distribution or blood pooling. This is also crucial for determining the postmortem interval and enables experts to gather critical evidence despite the severe state of decomposition *(Cartocci et al., 2019; Levy, Harcke, Mallak, 2010; Hussein et al., 2022; Wagensveld et al., 2017; Klein et al., 2015, Shen et al., 2024; De-Giorgio et al., 2021; Wang, Zheng, Zhang, Ni, Zhang, 2017)*

In the case of burnt bodies, postmortem imaging is invaluable because of its ability to penetrate charred and damaged tissues, which are often fragile and difficult to handle during traditional autopsy. Burn injuries

typically destroy the soft tissues and outer layers of the body; however, postmortem imaging can still capture detailed images of bones, remaining internal structures, and any foreign materials. Postmortem imaging is essential to exclude the cause of death in burned corpses, especially when foul play is suspected. In severely burned corpses, determining the cause of death can be particularly challenging because of the extensive damage inflicted on both the external and internal structures of the body. CT scans are particularly effective in such cases, as they can detect and differentiate between thermal and traumatic fractures, assess the extent of damage to the skeletal system, and assess the state of internal organs and other critical structures that might still be preserved despite the burns. Imaging can help detect signs of pre-existing medical conditions, such as heart disease or stroke, that may have caused death before the fire. Additionally, it can reveal other key indicators, such as the presence of fire products in the airways, which helps determine whether the individual was alive when the fire started or if the individual was already deceased *(Aydoğdu et al., 2021; Coty et al., 2018; De Bakker, Roelandt, Soerdjbalie-Maikoe, Van Rijn, De Bakker, 2019).*

In cases of dismemberment, images of dismembered limbs and organs enable forensic experts to analyze the methods and tools used for dismemberment. Imaging can reveal the characteristics of the cuts or breaks in bones, such as whether they were made with a saw, knife, or another sharp object, which can help investigators link the crime to a specific weapon or perpetrator. Postmortem imaging is particularly useful when multiple body parts are discovered at different times or locations because it enables forensic teams to compare anatomical structures and confirm that the parts belong to the same individual *(Matzen, Ondruschka, Fitzek, Püschel, Well, 2022; Maiese et al., 2020)*

Overall, in cases involving decomposed or burned bodies, postmortem imaging is a critical tool that provides comprehensive insights into the cause of death, aids in identification and preserves valuable forensic evidence that might otherwise be lost. Imaging also preserves digital

records of body parts for future reference, ensuring a comprehensive forensic investigation.

Examinations of Pediatric and Neonatal Deaths: Postmortem imaging is particularly beneficial in cases involving pediatric or neonatal deaths, where the size and fragility of the body make traditional autopsy challenging. Imaging technologies such as DR, CT, MRI, ultrasonography (US), and even mammography due to its magnification feature, allow forensic specialists to examine the body in detail without the need for dissection.

Postmortem imaging is highly effective for detecting congenital and developmental anomalies or undiagnosed medical conditions that may have contributed to death in neonates and young children. Postmortem DR provides information about the skeleton, particularly regarding skeletal dysplasia diagnosis. Postmortem MRI is especially useful for assessing soft tissues, providing a clearer view of potential causes, such as malformations, congenital anomalies, hemorrhages, and infections *(Ashby et al., 2022; Arthurs, Van Rijn, Taylor, Sebire., 2015; Gould et al., 2019; Thayyil, Robertson, Sebire, Taylor, 2010)*

In cases of sudden infant death syndrome (SIDS), postmortem imaging can exclude the cause of death and provide critical insights into potential explanations, for instance, subtle abnormalities in the airways, lungs, or heart that might indicate suffocation, infection, a congenital issue, or ruling out traumatic injuries or signs of abuse (*Van Goethem et al., 2024).*

In suspected cases of child abuse and shaken baby syndrome (SBS), both antemortem and postmortem CT and MRI are particularly useful for detecting internal injuries like organ damage, hemorrhages and other traumatic injuries *(McGraw, Pless, Pennington, White, 2022; Cartocci et al., 2021)*

Postmortem imaging also allows for repeated analysis and the opportunity for pediatry, radiology, and pathology specialists to collaborate on a case, providing a multidisciplinary approach to understanding the cause of death. Overall, postmortem imaging in pediatrics and neonates provides enhanced information to clarify the cause of death, making it an essential

tool in modern pediatric forensic investigations.

Examinations of Asphyxiation Cases: Postmortem imaging plays a crucial role in the forensic investigation of asphyxiation cases, such as strangulation and drowning, by providing noninvasive insights into the internal and external injuries associated with these types of death.

In cases of strangulation, CT and MRI scans are valuable for examining the neck and throat anatomy. CT and MRI scans can provide detailed images of internal hemorages in the neck muscles, arteries, and veins, which are often key indicators of manual or ligature strangulation. CT scans, in particular, can provide bone and cartilage damage in the neck and throat, allowing forensic experts to assess damage to the larynx, trachea, and hyoid bone, which may have been fractured during strangulation. Additionally, 3D reconstruction of CT images can help detect external injuries, such as ligature marks around the neck. By identifying these internal signs of trauma, postmortem imaging can corroborate evidence of strangulation, even in cases in which external bruising or marks are minimal *(Gascho, Heimer, Tappero, Schaerli., 2019; Deininger-Czermak, Heimer, Tappero, Thali, Gascho, 2020; Nagai et al., 2024; Yen et al., 2005)*

In cases of drowning, CT scans reveal key physiological changes associated with water inhalation. CT imaging of the lungs can reveal fluid in trachea, bronchi, pleural effusion, pulmonary emphysema, frothy fluid or debris in the lungs, as well as gastric and duodenal dilatation, fluid in the sinuses and pericardial effusion—findings that are characteristic of drowning. It can also help distinguish drowning from other causes of death by analyzing fluid accumulation in the sinuses, airway obstructions, and water in the stomach due to drowning *(Van Hoyweghen, Jacobs, Op de Beeck, Parizel, 2015; Ogawara, Usui, Homma, Funayama, 2022; Wang et al., 2020, Plaetsen et al., 2015)*

By providing a noninvasive, detailed examination of the body's internal structures, postmortem imaging enhances the ability of forensic experts to detect subtle signs of asphyxiation, ultimately contributing to

more accurate cause-of-death determinations in cases of strangulation and drowning.

Examinations of Violent Deaths, Homicides, and Abuse: In cases of gunshot, stab, or blunt force trauma, imaging can identify internal injuries without the need for dissection.

In firearm and gunshot injuries, postmortem imaging offers a detailed, noninvasive method to examine and document the number and exact anatomic location of the bullets, bullet fragments, or shrapnel. Imaging is especially useful for determining the trajectory of bullets and the extent of tissue damage *(Van Kan, Haest, Lahaye, Hofman, 2017; Usui et al., 2016)*

CT scans are particularly valuable in firearm and gunshot injuries due to their ability to produce high-resolution, high-contrast, three-dimensional, cross-sectional images, allowing forensic experts to trace the trajectory of bullets, bullet fragments, or shrapnel and assess the injuries caused along their trajectory. CT imaging can also detect and localize small metallic fragments, which are often dispersed within the body after a gunshot, providing crucial evidence in reconstructing the events of the shooting, determining the angle of the missile trajectory, and even information about shooting distance and determining the caliber of the bullet based on the size and shape of fragments left in the body *(Oehmichen et al., 2003; Junno, Kotiaho, Oura, 2022; Alves et al., 2020, Woźniak, Moskała, Rzepecka-Woźniak, 2015).*

In puncturing, penetrating, and perforating injuries, postmortem imaging provides information about the depth, trajectory, and impact of these injuries on internal organs and structures. For perforating injuries where the object fully penetrates the body, postmortem imaging provides critical insights into both entry and exit wounds and internal damage caused along the trajectory *(Ali, Mourtzinos, 2022; Schnider et al., 2009; Woźniak et al, 2015).*

In the investigation of abuse cases, DR, CT, and MRI can reveal different aspects of injuries. For example, DR and CT scans can clearly show fractures, especially those in delicate areas, such as the skull, ribs, and long bones, which are often

associated with physical abuse. Additionally, these imaging modalities can identify patterns of bone healing, such as multiple fractures occurring at different stages of healing, which are indicative of repetitive trauma and a key marker of chronic abuse (*Woźniak et al., 2015; Van Wijk, Vester, Arthurs, Van Rijn, 2017).* MRI is particularly useful for detecting soft tissue injuries, including internal hemorrhage and organ damage, which may result from shaking, blunt force trauma, or other forms of abuse *(Hart, Dudley, Zumwalt, 1996).*

The digital nature of postmortem imaging allows for detailed documentation and preservation of evidence, repeated examinations, and consultations with forensic experts, ensuring thorough and accurate analysis of evidence. Overall, postmortem imaging is crucial for understanding the full extent of violent deaths, homicides, and abuse, enhancing forensic investigations by providing precise, detailed, and noninvasive visualizations of internal damage.

Examination of Accidental Deaths: Motor vehicle accidents, falls from height, and other traumatic incidents often result in complex injuries. Postmortem imaging can capture the extent and nature of trauma, fractures, dislocations, internal hemorrhage, and organ damage, enabling investigators to reconstruct the sequence of events leading to fatal injuries *(Obertová et al., 2019; Wijetunga et al., 2020; Jalalzadeh et al., 2015).*

Postmortem imaging may provide information for understanding the biomechanics of injury. For instance, analyzing the forces exerted on the body during a crash or fall can help forensic experts determine factors like the direction and intensity of the impact *(Fukuda et al., 2024).* Another example is the distribution and severity of injuries, which can provide information to determine whether the victim was a driver, passenger, or pedestrian and whether the injuries were consistent with the vehicle's speed, point of impact, or position of the body at the time of the crash (*Breen, Næss, Gaarder, Stray-Pedersen, 2021; Moskała, Woźniak, Kluza, Romaszko, Lopatin, 2017).*

Additionally, injuries like whiplash or dashboard injuries help forensic experts reconstruct the accident and provide insights into how the collision occurred and the likelihood of survival based on injury patterns *(Johansson, 2006; Van Goethem, Biltjes, Van Den Hauwe, Parizel, De Schepper, 1996; Aiker et al., 1975).*

In legal contexts, postmortem imaging is especially useful when there are conflicting eyewitness accounts or when a detailed biomechanical analysis is required to understand the accident's cause. Furthermore, postmortem imaging helps identify additional factors that might have contributed to the accident, such as pre-existing medical conditions, which can be detected through scans and linked to sudden incapacitation, such as heart attacks or strokes. Additionally, postmortem imaging enhances the accuracy and depth of forensic investigations, providing clear, comprehensive insights that help reconstruct the accident, identify contributing factors, and support the legal processes.

Examination of Clinical Research and Missed Diagnoses: Postmortem imaging is a noninvasive method for investigating and understanding medical conditions that may have been overlooked or misinterpreted in cases in which missed diagnoses are suspected or in which further clinical research is essential. In cases in which the patient's symptoms were not fully understood or properly diagnosed, postmortem imaging allows for retrospective investigation and provides crucial insights into conditions that may have gone undetected during clinical care *(Sonnemans, Kubat, Prokop, Klein, 2018; Inai et al., 2016; Roberts et al., 2012).*

These imaging studies can help identify the missed condition and its evolution, providing a better understanding of disease progression and patient outcomes *(Kolasinski et al., 2012).*

Postmortem imaging is essential for improving diagnostic accuracy for future patients because researchers and clinicians can learn from these postmortem findings to refine clinical practices and enhance early detection strategies. Postmortem imaging is crucial for quality assurance and medical education because it allows healthcare institutions to investigate potential diagnostic errors. It also

enables clinicians and pathologists to review complex cases in which the cause of death is unclear, thereby facilitating a more detailed analysis of treatment efficacy and medical decision-making. Additionally, digital records produced by postmortem imaging can be stored and shared for further research or educational purposes, thereby contributing to spreading knowledge to improve patient care and prevent similar diagnostic errors in the future. Overall, postmortem imaging plays a pivotal role in clinical research by helping uncover missed diagnoses, enhancing medical knowledge, and driving advancements in diagnostic and treatment strategies.

3. Postmortem Imaging Modalities

Postmortem imaging encompasses various radiological modalities, such as ultrasonography, digital radiography, fluoroscopy, angiography, computed tomography, and magnetic resonance imaging, that provide different types of information about the body in question. Each modality has strengths and applications in forensic analysis, which are briefly described below.

Postmortem Ultrasonography (PM-US):

Postmortem ultrasonography is a modality that uses sound waves to examine the internal structures of the body after death. It serves as a supplementary diagnostic technique to the traditional autopsy, allowing for the visualization of organs, tissues, and any potential pathological changes. This method is particularly useful in forensic investigations to determine the cause of death, identify trauma or hemorrhage, and detect signs of disease. It also has applications in pediatric and perinatal cases (*Shelmerdine, Sebire, Arthurs, 2021; Shelmerdine, Sebire, Arthurs, 2019 (a); Shelmerdine, Sebire, Arthurs, 2019 (b)).* PM-US can be considered a safer alternative to high-risk postmortem procedures, particularly in case of infectious diseases, where minimizing exposure risks is paramount. *(Kanchan, Shrestha, Krishan, 2021).* PM-US can detect pathological findings like cardiac hypertrophy, pericardial tamponade, aneurysm of the abdominal aorta, pleural effusions, subphrenic abscess, ascites or intra-abdominal bleeding, liver metastasis and cirrhosis, fatty change of liver, parenchyma, bile

stones, renal cysts, diverticulum of the urinary bladder, prostate hyperplasia, myoma uteri, intracranial hemorrhage in infants, bone fractures, and implants in breasts *(Uchigasaki, 2006).*

Postmortem Digital Radiography (PM-DR): PM-DR is one of the x-ray imaging modalities in postmortem imaging and is particularly valuable for visualizing bones, dental structures, foreign objects like bullets, shrapnel, smuggling packages, and medical devices.

PM-DR is widely used in forensic cases involving trauma, dismemberment, skeletal fractures, or joint dislocations *(Hughes-Roberts, Arthurs, Moss, Set, 2012)*

PM-DR is also beneficial in perinatal cases, as it aids in estimating gestational age and intrauterine growth by detecting ossification centers and measuring the length of long bone shafts *(Fuente, Dornseiffen, Noort, Laurini, 1988; Shelmerdine, Arthurs, 2023; Shelmerdine, Sebire, Arthurs, 2021)*

DR is especially useful for identifying victims by crosschecking antemortem and postmortem dental records *(Viner, Robson, 2017; Heinrich, Güttler, Schenkl, Wagner, Teichgräber, 2020).*

Although PM-DR lacks detailed soft-tissue visualization and superposition problems due to its 2D nature, it remains an essential tool for fast and efficient skeletal analysis and detection of foreign objects in forensic investigations.

Postmortem Fluoroscopy (PM-F): PM-F is a dynamic imaging technique that provides real-time x-ray images. PM-F is useful for whole-body scanning to evaluate skeletal structures, foreign bodies, and medical devices like in PM-DR. Although PM-F has limited soft tissue resolution, its ability to provide continuous dynamic imaging makes it a unique and important technique for certain postmortem examinations. For example, real-time visualization capability makes fluoroscopy a valuable tool for guiding procedures, such as locating foreign objects like bullets, projectiles, or shrapnel, in the body and assisting in the retrieval of these items without extensive dissection.

Postmortem Angiography (PM-A): PM-A is a specialized fluoroscopy or computed tomography

technique in which contrast agents are injected into the vascular system to visualize blood vessels and provide detailed images of the circulatory system in deceased individuals. This technique is particularly useful in cases of suspected vascular abnormalities or trauma, such as aneurysms, vascular blockages, hemorrhage, varices, and traumatic injuries. Additionally, it is critical for detecting small ruptures or microbleedings that may not be visible with conventional autopsy methods. By providing a clear visualization of the vascular system, PM-A helps forensic experts understand the cause and mechanism of death in cases in which vascular trauma or disease play a significant role *(Grabherr, Djonov, Yen, Thali, Dirnhofer, 2007; Grabherr et al., 2018, Ross et al., 2014; Franckenberg, Flach, Gascho, Thali, Ross, 2015).*

Postmortem Computed Tomography (PM-CT): PM-CT is one of the most effective X-ray imaging techniques, offering high-resolution, high-contrast, three-dimensional, cross-sectional, and reconstructed images of the body.

PM-CT scans are particularly valuable for viewing fractures, hemorrhages, internal damage, and the presence and trajectory of foreign objects *(Adelman et al., 2018; Willaume et al., 2018; Rutty, 2020; Burton, Kitsanta, 2020)*. Additionally, PM-CT is essential in mass disaster situations where multiple bodies need to be quickly scanned for victim identification *(Brough, Morgan, Rutty, 2015; Sidler, Jackowski, Dirnhofer, Vock, Thali, 2007)*. PM-CT scan is also crucial in pediatric and neonatal cases because it can detect subtle injuries or congenital abnormalities *(Gould et al., 2019; Thayyil, Robertson, Sebire, Taylor, 2010)*. PM-CT can be used as a replacement to invasive autopsy practice in the investigation of high-risk autopsies, such as in cases of infectious diseases *(Roberts, Traill, 2021; De-Giorgio et al., 2021; Filograna et al., 2022).*

The digital nature of CT images allows the post-processing, reconstruction, and measurement of different parameters on the target image, such as distance, area, volume, and density. It also allows for easy and long-term storage, enabling ongoing analysis and collaboration with other forensic experts, making it one of the most comprehensive and reliable

imaging techniques for postmortem investigations.

Postmortem Magnetic Resonance Imaging (PM-MRI): MRI is a non-ionizing imaging modality, unlike techniques that use X-rays for imaging.

MRI is excellent for detecting subtle tissue changes, such as brain hemorrhage, edema, infarcts, tumors or neurodegenerative conditions that may have contributed to death even in putrefied bodies *(Bauer, Berger, Gerlach, Scheurer, Lenz, 2022; Saito et al., 2024; Ringger, Schwendener, Klaus, Jackowski, Zech, 2023)* In pediatric and neonatal deaths, MRI is invaluable for identifying congenital anomalies, brain malformations, and undetected infections *(Arthurs et al., 2015; Pérez-Serrano et al., 2021; Addison, Arthurs, Thayyil, 2014; Thayyil et al., 2013)* In addition, it plays a critical role in forensic investigations involving strangulation, suffocation, and other asphyxiation deaths by visualizing soft tissue injuries in neck and airway structures *(Gascho, Heimer, Tappero, Schaerli, 2019; Deininger-Czermak, Heimer, Tappero, Thali, Gascho 2020; Yen et al., 2005)*

MRI provides exceptional soft-tissue contrast, which is particularly useful for examining internal organs. Although MRI is less efficient than CT for skeletal analysis or cases involving foreign objects, it remains an essential tool for investigating deaths where soft tissue pathology is the primary concern.

Conclusion

Postmortem imaging has become a critical adjunct to conventional autopsy due to its ability to offer detailed, noninvasive insights into the deceased's internal anatomy that complement the findings of traditional dissection. While conventional autopsy remains the gold standard for determining the cause of death, it can be limited in certain scenarios, such as when disintegrated, decomposed, or burned bodies are involved or pediatric or neonatal deaths occur, where the size and fragility of the body make traditional autopsy challenging.

Postmortem CT or MRI provide three-dimensional and cross-sectional images of the body, allowing for an in-depth examination of fractures, dislocations, tissue damage, organ abnormalities, or the presence of

foreign objects. This makes postmortem imaging an essential tool in forensic and medical investigations, where data accuracy and precision are paramount. Additionally, postmortem imaging can detect certain subtle pathologies that may be missed or misinterpreted during traditional autopsy. For example, microfractures, gas embolisms, or small brain lesions may not be easily visible during manual dissection but can be effectively captured and analyzed through imaging.

Postmortem imaging also allows for the repeated review of the same evidence without further invasive procedures, which is especially beneficial in complex cases that require multidisciplinary consultation. Furthermore, when time is critical, such as during mass casualty events, imaging is a faster alternative that can quickly assess multiple bodies. Another key benefit of postmortem imaging is the ability to store digital images for future reference, which makes it possible to perform retrospective crosschecking. Additionally, postmortem imaging allows forensic experts to examine missed diagnoses retrospectively and contributes to a deeper understanding of medical conditions, improving forensic and clinical outcomes.

Combining postmortem imaging with conventional autopsy not only ensures a more comprehensive investigation but also provides a higher level of accuracy, helping to rule out or confirm certain diagnoses or causes of death that might otherwise remain ambiguous. This integration is particularly crucial in modern forensic practice and precise medical examinations. Thus, postmortem imaging is a must-process alongside conventional autopsy to enhance diagnostic accuracy and to provide a complete understanding of the circumstances surrounding death. As technology continues to advance, the role of postmortem imaging in death investigation and research will only become more significant, providing an essential complement to traditional autopsy methods and advancing the field of forensic medicine and pathology.

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NOVEL TECHNIQUES USED IN IDENTIFICATIONS THROUGH DENTAL STRUCTURES: THE ROLE OF FORENSIC ODONTOLOGY IN CRIME SCENE

Mehmet Ali KILIÇARSLAN

Chapter 5

Novel Techniques Used In Identifications Through Dental Structures: The Role Of Forensic Odontology In Crime Scene

MEHMET ALİ KILIÇARSLAN[1]

The Relationship between Forensic Sciences and Dentistry

A crime scene is defined as the area where the manner in which the acts defined as crimes by law were committed and the relationship between the victim and the accused can be determined. Edmond Locard said, "every contact leaves a trace" to describe the dynamism of the crime scene. In fact, not only the physical traces but also the emotional traces in the contacts realized through communication affect the mental state of the person and carry much forensic evidence. However, since physical traces can be compared with concrete data, they can undoubtedly reveal more rational results than emotional traces.

In the field of criminalistics, fingerprints are undoubtedly the most important data due to the richness of the data stored in the database as well as its uniqueness, but in relation to dentistry, lip prints, teeth or bite marks, tongue prints and palatal rugae prints are also used to identify individuals. The idea of using teeth as an aid in identification was first accepted and implemented at the meeting of the Dental Society in Paris in 1887 (Kılıçarslan, 2024). Cuban-born Dr. Oscar Amoedo, also called the "Father of Forensic Dentistry", published a paper in 1897 describing the identification of victims of a fire using teeth (Mânica et al., 2019). Forensic dentistry, also called forensic odontology, is a field of dentistry that comes through the proper management, examination, evaluation

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and presentation of dental information, documents and details for the benefit of justice in criminal or legal processes (Prajapati et al., 2018).

In cases of death, accident, crime, abuse or violence, which are suspicious and forensic events that need to be clarified, the clarification of the case by using the knowledge and methods of all specialities of dentistry is among the job descriptions of the field of study referred to as Forensic Dentistry or Forensic Odontology. This case clarification may be the analysis of an incident or the identification of the victim, victim, suspect or criminal. In every case, it is impossible to carry out identification using traditional methods. In cases where traditional methods are insufficient, alternative methods that have recently emerged in forensic sciences come into play (Hancı, 2002; Harorlı, 2006; Karakuş, 2011; Kılıçarslan and Demir, 2023).

Areas where dentistry can support forensic issues:

1- Documentation and storage of dental treatment records for malpractice litigation and identification (Devadiga, 2014).

2- Identification by comparing ante-mortem and post-mortem dental information in disasters with mass death cases (Berketa et al., 2017).

3- Identification and collection of bite marks, palate marks, lip marks or cheek and nose marks on living or inanimate objects (Gupta et al., 2014).

4- Reporting symptoms and signs of partner violence, elder abuse, and child abuse to the competent authorities (Afşin, 2004).

5- Assessing dental evidence and providing expert witness services in cases of identification, bite mark analysis, malpractice, fraud and violence (Devadiga, 2014).

6- Determination of a person's age in cases such as punishment, marriage, and starting school (Afşin, 2004).

7- Determining the sex of the person (Tsuchimochi et al., 2002).

8- Detection of intraoral trauma and injuries (Afşin, 2004; Gupta et al., 2014).

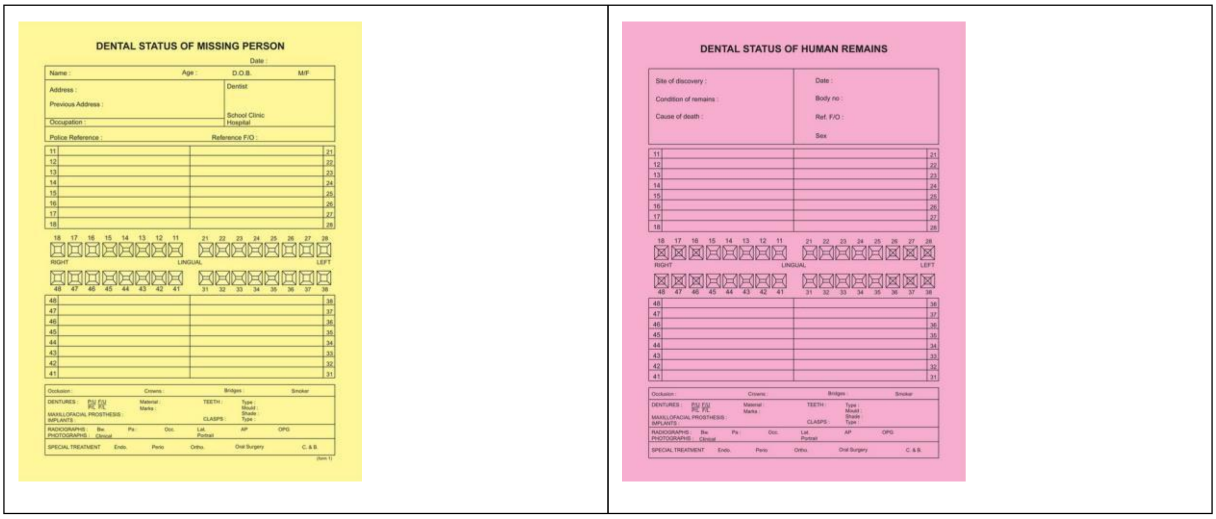
Identity is defined as the total of morphological, physiological and physical characteristics that make an individual unique, being a set of functional or psychic, normal or

pathological physical characteristics that define the individual (Araujo et al., 2019). In forensic investigations, identifying victims, deceased individuals, victims of crime, suspects, or perpetrators plays a crucial role (Er, 2023). As challenging as it is important and requires meticulous attention, this process can lead to irreversible consequences if errors occur. It combines various procedures during the identification process to uniquely associate a person or object with specific characteristics. There are many techniques for personal identification. These techniques can include DNA comparisons, body print analysis, comparison of dental records, use of antero-posterior metric data, retinal scanning, age or sex determination, and many more. Since a combination of different techniques may need to be used to make an identification or identification, it is often inevitable that other supporting evidence, such as clothing, body tattoos or piercings, eyewitnesses, documents, and objects, will be used for personal identification (El-Domiaty et al., 2010; Kılıçarslan 2024). Rapid and accurate identification of unidentified bodies is important both from a legal point of view and from social, religious, and moral issues. It is also important to immediately return the remains to families for mourning purposes (Michalski, Malec, Clothier and Bassed, 2024).

The identification and identification procedure, which is defined as the recognition, identification and differentiation of individuals from other individuals, nowadays includes the necessity of "identity verification" processes, that is, the association of the person with the identity that the person has, due to the increasing world population, imbalances in income distribution around the world, intensive and widespread population mobility, and increased global travel opportunities (Kılıçarslan and Ağır, 2023). Identification constitutes one of the most important areas of forensic dentistry studies, as teeth and saliva directly reveal important determinants of the individual through morphological and chemical analysis and genetic tests (Kılıçarslan et al., 2023).

Recovering human parts, identifying the deceased, and documenting the cause and manner of death are the main objectives of disaster victim identification (DVI) operations. In order to develop

preventive measures, DVI-related investigations may provide assistance by reconstructing the cause of the event. In 1984, the first written DVI guidelines were introduced by the International Police Organization (INTERPOL), and these guidelines are regularly being updated by four scientific sub-working groups on forensic specialities: Ridgeology (Fingerprints), Anthropology, Dentistry, and Molecular Biology (DNA). INTERPOL DVI Manual and the accompanying documentation forms are generally used worldwide for DVI operations as the manual and forms provide a standardized framework for managing mass fatality incidents (Cordner and Ellingham, 2017; INTERPOL, 2018).


DENTAL STATUS OF MISSING PERSON

DENTAL STATUS OF HUMAN REMAINS

Figure 1: Dental part of the Interpol DVI guide

Forensic Dentistry at the Crime Scene and in the Identification Process

Teeth and Dental Restorations

Teeth consist of the crown, macroscopically visible in the mouth, and the root parts in the jawbone. The hard tissue of the teeth consists of the enamel covering the crown, the cementum covering the root, and the dentin located underneath them. Enamel surrounds the crown part of the teeth, which is visible in the mouth from the outside. It is the hardest tissue in the body. It is thickest at the cutting edge of the anterior teeth (2 mm), the tubercle tops of premolars (2.3-2.5 mm) and the tubercle tops of molars (2.5-3 mm). It is reset at the border of the junction with cement. Its structure

comprises 95-98% inorganic, 1% organic material and 4% water. It contains 90% hydroxy apatite crystals by volume. The root canal contains the dental pulp and is surrounded by dentin. Usually, an adult has 28 to 32 teeth, and each tooth has 4 to 5 surfaces. The first anterior tooth is called the central, the second anterior tooth is called the lateral incisor, and the third tooth is called the canine or canine. Premolars are also called premolars, and molars are also called molars. From a paleontological point of view, there are also specific anatomical elevation designations for the upper and lower permanent molars. There is no distinction in these designations for the right and left jaw (Turner and Scott, 1997). All these naming and numbering systems apply to both primary and permanent teeth (Harorlu, 2006; Demir, 2018). In addition, many kinds of materials are used in dentistry treatments. Therefore, there are also different features that have been acquired by human hands through teeth. According to these factors, the probability of an overlap between the dental findings of one person and another is significantly low. Thus, personal identification through dental findings can be considered useful for closed disasters. Even in the absence of documented dental treatment, in some cases, identification is possible through photographs and descriptions.

A neonatal line is formed when primary teeth fail during birth. This line provides information on whether the individual was born vaginally or by caesarean section. It is important information for forensic sciences in the identification of infants and children (Hurnanen et al., 2017).

The most used tooth numbering systems are the Palmer System, the Haderup (European) System, the American (ADA) System and the World Dental Association (FDI) System, which is also widely used in our country. In the FDI System, the upper right half jaw is numbered as 1, the left maxilla as 2, the left half of the mandible as 3, and the right mandible as 4. The tooth number is then indicated (Kılıçarslan, 2024).

Teeth are the most resilient part of the human body and can remain almost intact for many years after death. They are biologically stable and retain information within their hard tissues that can serve as markers of the individual's physiological and

pathological events throughout life. Additionally, any dental treatments, such as restorations and prostheses, performed by a dentist alter the structure of the teeth in a way that can make them uniquely identifiable. Thus, while identifying an unknown corpse, evaluation of biological and chemical findings in one's teeth plays a crucial role. Because of the anatomy and morphology, the only preserved body remnant in burn cases can be teeth, making identification through dental records a highly important tool for forensic work (Berketa et al., 2017).

The diagnostic significance of teeth lies in highly mineralized composition of teeth, which makes them resistant to external environmental factors. They do not undergo significant changes due to post-mortem decomposition and are typically resistant to flames, alkalis, and even mild acids. However, since teeth from burnt human remains are extremely fragile, preservation of teeth for evidential purposes has become a major problem. In this sense, the fragility of the remains must be taken into account when dealing with burnt tissue remains. Intense fires also cause the pulp to burn and the dental crowns to burst. This will cause any tooth that could be used to identify the victim to break away from the gingival margin, and only the root can be assessed as the insulating properties of the upper or lower jaw protect the roots. Therefore, the assistance of a forensic dentist is usually sought to preserve the burnt tooth structures before any procedure is undertaken. When dealing with burnt tooth remains, a systematic approach must be followed to prevent the loss of potential dental evidence and preserve the tooth's structural integrity at every stage of the assessment. Furthermore, this protective feature would also make it possible to obtain Deoxyribo Nucleic Acid (DNA) samples from tooth tissues (mitochondrial DNA analysis from dentin, nuclear DNA analysis from pulp) or tooth peripheral tissues and deliver them to the expert who will perform DNA testing (Reesu, Augustine, and Urs, 2015).

Identifying various restorative materials used in the mouth for therapeutic or restorative purposes, which are resistant to varying temperatures, provides crucial post-mortem clues, especially when pre-mortem records are available. For example, amalgam can withstand

temperatures up to 800°C. Base metal alloys have melting points between 1275°C and 1500°C and do not deteriorate up to these temperatures. Dental porcelain, on the other hand, can remain intact up to these temperatures because it is sintered at temperatures above 1000°C. However, tooth-colored composites, which have become very popular in recent years, can be difficult to identify both clinically and radiographically, and this can be difficult even for forensic odontologists to detect. Various methods have been used to determine the presence of these restorative materials during autopsy examinations. Among these methods, dyes that reveal bioplaque, trans-illumination and quantitative light-source fluorescence techniques play an important role. In addition, when a tooth is exposed to extreme temperatures, the dehydration that develops in its structure causes it to shrink and disintegrate, leading to the displacement of the restorative material on it (Brandao et al., 2007).

Tooth decay

Dental caries are cavities formed on the enamel, dentin, and cementum surfaces of the tooth due to the effect of acid-producing bacteria on bacterial plaques. Caries can be observed on the crown or root surface of the teeth (Yaşar and Erol, 2007). Caries may be shadowed on radiography because the restorations are mostly light-colored (radiopaque). Smooth radiolucency borders increase the likelihood that the area seen is a restoration (Bilgen, 2016).

Dental anomalies

Pathologies are of great importance for dental identification. In forensic dental comparisons, some pathologies include the comparison of some important individual features, such as the absence of teeth, excess of certain teeth, a diastema, deformities, abrasion, coloration. All these anomalies help in comparing pre-mortem and post-mortem matches in dental identification and confirm the identity of an individual (Krishan et al., 2015).

Mutilations and marks on teeth

Mutilation is a Latin word that means to distort or change the structure. Various carving and scraping operations on the tooth structure are examples of mutilation. In some cases, even tooth extraction is considered mutilation (Yaşar and Erol, 2011).

Teeth are massive and fragile structures and can be altered sometimes due to certain professional habits. For example, tailors who pass thread through cotton between their teeth before threading a needle often exhibit characteristic notches on the incisal edges of their teeth. It is said that shoemakers holding small studs or tacks in their mouths before hammering them into the edges of shoes, likewise carpenters biting studs and tailors biting needles, can create distinctive wear patterns on the teeth. Similar characteristics can be seen in societies that consume excessive sunflower seeds. It is important that personnel working in battery production may have acidic demineralization in their front teeth due to the materials used, and all such features may provide some clues to the identity of the unidentified person (Reesu, Augustine, and Urs, 2015).

Anatomical Structures in the Orofacial Region

Teeth affect pathologies of surrounding tissues such as gingiva, jawbone, mucous membranes, tongue, salivary glands, jaw joint, paranasal sinuses, and soft and hard tissues. These pathologies may appear radiopaque (RO) or radiolucent (RL) on radiographs. Foreign bodies in the surrounding tissues of the teeth are observed in radiographs in various localizations in the paranasal sinuses or in the alveolar bone, depending on their density (Yaşar and Erol, 2007; Demir, 2018).

Orofacial Traces Important for Forensic Sciences

Tooth and bite marks

A bite or teeth mark is evidence left on the object between the jaws when a living creature brings the lower jaw closer to or closes the upper jaw by applying a certain amount of pressure. Bite mark analysis is a common method of crime evidence detection that deals with the examination and comparison of marks left by human teeth or bites on skin, food, and other objects at the crime scene. The purpose of bite or teeth mark analysis is to determine whether the bite mark is specific to a person, providing evidence that can be used in court (Mohammad, Ahmad, Kurniawan, and Yusof, 2022). These analyses depend on the fact that teeth marks can find specific tooth patterns, such as alveolar arches patterns or multiple overlapping blue, green, and

black marks (Maji et al., 2018). Additionally, bite marks can appear differently due to the elasticity and flexibility of the skin. The appearance of bite marks can vary depending on the force of the bite, the structural characteristics of the bitten object, the location of the bite on the body, and the angle made by the maxilla and mandible at the time of the bite. (Van der Velden et al., 2006; Osman et al., 2017). In forensic science, a bite mark is defined as a patterned injury created by structures related to human or animal teeth. Typically, the six anterior teeth in humans produce indentations that are visible in bite marks. Analyses based on individual dental characteristics can be used to identify the person responsible for the bite (Fournier et al., 2020; Kılıçarslan et al., 2023).

Palatoscopy

Palatal rugae are irregular, asymmetrical ridges or elevations of tissue folds on the anterior 1/3 of the palatal mucosa just behind the upper incisors on both sides of the median palatal raphe (Smriti et al., 2021). It is known that there are more prominent rugae on the left side of the jaw and in males. (Simmons et al., 1987; Er, 2023). Palatoscopy or rugoscopy refers to the study of palatal rugae for the purpose of identification (Poojya et al., 2015). Antemortem records are required for palatal rugae to be used in forensic cases (Shukla et al., 2011).

Tongue prints

The tongue is a vital internal organ located in the oral cavity, well protected by the hard palate, cheeks, lips and teeth, showing structural differences from individual to individual (Zaidi et al., 2013). Color, shape, and surface features are characteristic of each individual and have become a tool for identification in biometric examinations. These features also show sexual dimorphism. The tongue may leave traces on the surfaces it meets. These marks can also be considered forensic findings (Kılıçarslan and Ağır, 2023).

Lip prints

Various elevations, depressions, grooves, and lines on the outer surface of the lip form the lip print. Lip prints refer to the marks left by the anatomical structure of the lips on various objects, whether living or non-living, including personal items. Along with these traces, from time to time, tissue fluids such as

saliva or blood and residues of care or make-up products may also be smeared on the objects. Lip prints provide information not only in the identification process but also in defining the character and dynamism of the crime scene (Kılıçarslan and Demir, 2023).

Ear prints

Among all biometric organs of the face, the ear is one of the most important parameters that has been proven to have superiority in identification for both biometric and criminalistics examinations, with its characteristics that have a significant variability between individuals and do not change with age and facial expressions (Kılıçarslan and Ağır; 2023).

Cheek prints

These lines formed by the nerve network and sweat gland opening points (pores) in the lower skin layer on the skin, extending to the upper skin, are called "papillary lines". The liquid substances secreted from these pores enable the formation of marks on various surfaces that can be made visible with the help of special powders and chemicals. Cheek (face) prints or scars which are based on the principle of comparing these pores, are marks that can be formed by leaning on surfaces such as glass, doors, walls or the handle of a long-barreled weapon used in the incident. The analysis of the cheek and additional traces, such as scars in this area, is also extremely important for forensic sciences (Kılıçarslan and Ağır; 2023).

Expert Witness Process Related to Medical Malpractice

The concept of "permissible risk" in the legal literature is known as complication in health practices. Malpractice or malpractice, on the other hand, applies to every professional group or every job; it means "a professional person performing the work that he/she is obliged to do in a sub-acceptable, non-standard manner". Medical malpractice, or malpractice in the field of medicine, refers to the wrong treatment of a patient by a physician or other healthcare personnel in such a way that the patient is harmed or the wrong treatment of the patient and the wrong care of the patient. It is also a common legal process for dentists to serve as experts in malpractice cases (Kılıçarslan, 2024).

Abuse and Trauma Cases

Child abuse is generally defined as being subjected to behavior by an adult over a period that is not accepted in the child's culture. In addition, sometimes geriatric patients in need of care are also abused. Dentists can detect these cases of abuse during dental treatment of both children and older adults.

Age Determination from Teeth

The pattern of tooth eruption can give important information to determine the chronological age of a person. Human teeth have four different periods (Uzuner et al., 2017). These periods can be listed as the eruption of milk teeth at the second age, the eruption of two permanent incisors and the permanent first molars between the 6 and 8 ages, the eruption of all permanent teeth except the third molars between the ages of 10 and 12, and finally the eruption of the third molars older than age of 18 years (Holobinko, 2012; Köse, 2023).

Among the methods that examine the hard tissues of the tooth in age determination, it is known that the methods that evaluate dentin translucency are one of the credible methods for estimating the chronological age of the person. Sclerosis of the tubules on the cementum with age leads to the development of root dentin translucency, which starts at the apex of the root and then progresses coronally. Several studies have reported that dentin translucency increases with age. As a result, this method is reliable in people over the age of 20 who have had all their permanent teeth extracted (Mohammad, Ahmad, Kurniawan, and Yusof, 2022).

Age determination or determination from teeth can be done with tables created based on the calcification of teeth in the germ located in the lower and upper jaw bones in the fetus, infancy, childhood and young adulthood periods, according to the crown and root formation stages, tooth eruption into the mouth and root apex closure. However, the tables are insufficient in the adult period. Age determination methods have been created with regression formulas of the changes seen in teeth after eruption into the oral cavity. These changes are abrasions on the teeth, changes in periodontal tissues, root transparency,

and reduced pulp space. However, it should not be ignored that the changes that occur in teeth after eruption into the mouth vary among communities due to factors such as gender, ethnicity, eating habits, and skeletal anomalies. For this reason, regression formulas created based on the correlation between changes in teeth and age should be updated for each community (Patil et al., 2023; Çelik, 2021).

Sex Determination from Teeth

The shape and size of a jaw can be used for sex determination or determination (Guyomarc and Bruzek, 2011). In mass casualty cases where individuals are so severely injured that they cannot be visually identified, dental, jaw and skull remains have confirmed to be the most important factor for identifying victims. Radiographic images of the jawbones are readily available. Therefore, radiographic methods are the traditional way to estimate gender (Patil et al., 2018, 2020; Esmaeilyfard, Paknahad, and Dokohaki, 2021; Anuja et al., 2023; Bu et al., 2023).

Facial Reconstruction and Facial Identification

Since antemortem records for evaluation may be limited, unavailable, or hard to obtain (Caplova et al., 2018), alternative methods of identification may be valuable in providing clues and supporting the DVI process (Blau et al., 2023). Facial recognition has the potential to be one of these. While muscle tissue is replaced with various plastic materials for reconstruction, commercial off-the-shelf facial recognition technology is now used by many agencies, including law enforcement and border control, to identify and verify people (Michalski, Malec, Clothier, and Bassed; 2024). Government agencies have large databases of facial images used for driver's licenses, passports, and mugshots. Given the difficulty of accessing post-mortem data, some have used creative approaches such as making molds from live specimens or artificially altering facial images of living people to simulate traumatic injuries.

Identification of Disaster Victims from Teeth

According to the Disaster Epidemiology Research Center, a disaster is "a situation or event that exceeds local capacity requiring external assistance on a national or an international level; an unpredictable and often sudden event that causes extensive damage, destruction, and human suffering." Mass casualty disasters, classified as natural, accidental or criminal, may require even more resources to handle because of its extent. In order for a disaster to be entered into the database, either 10 or more people must be reported dead, 100 or more people must be reported affected, a state of emergency must be declared, or an international call for assistance must be made. Disasters can also be classified as open, closed or mixed disasters. Disasters such as earthquakes, tsunamis or floods are defined as open disasters, where the identities of the victims are usually not recorded locally. On the other hand, aircraft or other vehicle accidents and hotel fires, where personal records are kept, are examples of closed disasters (Below, Wirtz, and Guha-Sapir, 2009; Nuzzolese and Vella 2007; Prajapati et al., 2018; Utsuna, 2019). In recent times, disaster-related deaths have increased due to the variety of transportation vehicles and ease of access to them, the increase in terrorism and climate change. Therefore, the need for additional resources and developments for effective disaster management has also increased (Nuzzolese and Vella 2007). In such cases, where bodies are decomposed beyond recognition, forensic anthropological methods, fingerprint analysis, forensic odontology techniques, radiology and DNA typing can be used for identification. Disasters can be classically separated into two categories; "natural disasters" including meteorological, geological and biological events such as epidemics and "man-made disasters", which can be divided into sub-sections. These include man-made fires, industrial accidents, transportation disasters, administrative disasters (such as poor-quality construction), war and conflicts, terrorist events and CBRN disasters (chemical, biological, radiological, nuclear and explosive).

Forensic odontology has played an important role in identifying disaster victims through comparative analysis

(Woods, 2014). Forensic dentists are personnel trained to quickly and accurately identify disaster victims under bad conditions. When burns, decay, or severe trauma make it impossible to identify a victim by other means, dental examinations can still provide a definitive and accurate match. In the future, there may be nuclear, chemical, or biological contamination of humans, and forensic dentists will continue to receive training and be ready to provide assistance no matter what happens in the future (Miller, 2024).

Novel Techniques of Forensic Dentistry

Optical and Digital Scanners

Oral scanners are becoming increasingly popular in dentistry, enabling the creation of detailed 3D models of the oral cavity. In dentistry, intraoral and model scanners are used to take dental impressions for prosthetic design and provide accurate, editable, stable digital impressions. It has been shown that intraoral scanners can be used to take impressions of indented ridges on the skin, such as bite marks, followed by an optical scan of the suspect's dentition for a record and then comparing the two objects with 3D software (Kılıçarslan and Oğuz, 2023).

Intraoral scanners allow bite marks to be recorded quickly. The 3D images obtained in this way can be transferred. Bite marks can be compared with surface comparison software that allows direct analysis. Essentially, 3D analysis gives more objective results than other methods. It is important to note that dental experience is essential to achieve the best results (Reesu et al., 2020).

Imaging information (intraoral photographs and radiographs) plays an auxiliary role in dental identification. By the way, optical scanners have presented as a new image tool in recent years. In forensic sciences, much research is being done on techniques for analyzing the patterns obtained by optically scanning the surface of a corpse. This use would allow many dentists to participate in the identification work and ease the mental and physical burden on those sent to do the work in the field (Nakamura, Kasahara, and Hashimoto, 2022).

Computer-Aided Applications in Forensic Dentistry

Artificial Intelligence (AI) is a field of computer engineering that deals with information processing technologies, creates the capacity to learn and perform cognitive tasks through models and algorithms, and produces outputs such as prediction and decision-making on physical and non-physical issues. Innovative applications of AI have been proposed for use in different processes, including gender and age determination, characterization of bite marks, post-mortem interval prediction, and DNA interpretation. For these reasons, it is important to consider whether the results of AI can indeed replace, diversify or complement previously used methods of forensics (Moretti et al., 2017; Cantürk and Özyılmaz, 2018; Bewes et al., 2019).

In forensic odontology, AI technology has been put into practice while determining age and gender based on dental features, human identification and characterization of bite marks. The evaluation of dental images is currently done by specialized radiologists or dentists while determining age in forensics. However, to overcome the errors caused by the variability and subjectivity of the human eye, automatic staging techniques and related age-determination methods have been developed (Galante et al., 2023).

The application of AI-based methods in forensic odontology is a significant breakthrough as it is a technique that provides reliable information for the decision-making process in forensic sciences. Therefore, scientific studies have revealed that AI technology is currently highly effective in the fields of (Jeddy et al., 2017) human bite marks, (Jain et al., 2020) gender determination, (Divakar, 2017) age determination and (Johnson et al., 2018) comparison of dental records.

In research processes, non-metric methods are based on the presence or absence of a specific condition, such as morphological features. However, metric features such as dental measurements are more precise and less subjective, and they can generate quantitative 3D data from teeth or radiographs. Machine learning techniques and data mining algorithms are vital to uncovering data and explaining it. The results of these techniques generate data and models to

predict future behavior, which allows us to identify patterns. Data mining applications will also have positive consequences in terms of reconstruction for human biological profiling, enabling informed decision-making (Vodanović et al., 2007; Esmaeilyfard, Paknahad, and Dokohaki, 2021).

AI is increasingly permeating the lives of modern people and becoming pervasive in all areas, to a degree that is, in some ways, frightening. To better understand AI, it is important to clarify the distinctions between AI, deep neural networks, artificial neural networks, deep learning, machine learning, virtual reality, augmented reality, Metaverse and data mining (Vodanović et al., 2023).

As in forensic medicine, AI and other digital technologies are increasingly used in forensic dentistry. AI can be used to enhance forensic dentistry in a variety of ways, including (Khanagar et al., 2021; Vodanović et al., 2023).

Investigation and comparison of dental databases

AI can be used to search databases that can help identify individuals. AI can identify specific types of teeth on radiographs with a high degree of precision and reliability, independent of personal interpretations (Tuzoff et al., 2019). Research also shows the possibility of AI recognizing dental implant brands and types with high accuracy, again through radiographs (Kohlakala, Coetzer, and Bertels, 2022). AI can help forensic dentists analyze X-ray images of teeth and jaws in their identification processes. Artificial intelligence can be used to reduce the need for manual labor and increase the speed and accuracy of searching and matching processes in databases (Choi et al., 2022). In addition, AI can become an effective method by detecting the possibility of tooth decay through predictive analytics.

The International Police Organization (INTERPOL) has defined fingerprints, dental records and DNA analysis as the primary identification methods for disaster victims (INTERPOL, Disaster Victim Identification Guide, 2018). These

methods are mainly based on antemortem (AM) and post-mortem comparisons. In dentistry, AM data generally consists of descriptive clinical records, dental models, photographs and radiographs. On the other hand, PM data includes all dental information and images collected during post-mortem examination or oral autopsy (Gorza and Mânica, 2018). In such cases, computer-aided software has been developed to assist in the complex comparison process, providing an objective list of the best matches between available AM and PM data (Al-Amad et al., 2007; Adams and Aschheim, 2016). Data input in these software programs may vary depending on the dental identification system. In each system, identifiers are translated into codes and algorithms that are understandable only for certain matches. The effectiveness of the reconciliation relies on the discriminability of the available data from AM and PM morphological, therapeutic and pathological dental descriptor data (Chomdej, Pankaov, and Choychumroon, 2015; Franco et al., 2019; Fan et al. 2020).

Since each of the missing teeth, caries, fillings or dentures is unique to each individual and teeth are the most durable tissue in the human body, forensic dental examinations have become an important part of the process of identifying disaster victims. Identification processes in forensic dentistry are usually based on visual comparison of antemortem dental records and radiographs with post-mortem dental examination results. However, such traditional forensic dental examinations are risky due to the lack of widely accepted reference points between antemortem records and post-mortem examinations, and written dental records may be kept incorrectly, or data may be lost. Therefore, written forensic dental examinations are prone to omission or description errors. The best way to improve these traditional methods is to standardize and digitize them. Although forensic odontology has made progress with digital technologies and incorporated them into the process, digital technologies have not yet become widespread in this field due to several factors, such as the need for specialized equipment and high cost. With the development of digital technologies, the global digitalization of forensic odontology will make an important social contribution to

identification processes in the future (Matsuda, Miyamoto, Yoshimura, and Hasegawa, 2020).

Tooth and restoration identification

AI can help forensic dentists identify and match individuals based on their teeth and jaws by analyzing dental images such as radiographs. Panoramic radiographs provide the most useful diagnostic information by showing the teeth as well as the maxilla, mandible, and facial structures in a single plane image. In recent years, AI has also played a significant role in replacing laborious or time-consuming image-processing processes for dental identification. Using deep learning, identification such as caries identification or restoration identification has rapidly improved (Figure 2). Convolutional neural network (CNN) is another important method that stands out in the field of image. It is a particularly useful method for finding comparative patterns for image recognition, including radiological applications in general medical fields (Choi et al.).

These techniques have shown outstanding performance in automatically determining the number of teeth and using CNNs to detect natural teeth, dentures, treated root canals and implants. It may be possible to fully automate the entire process of identification by adding automatic calculation of detection information and building a large database of panoramic movies to effectively reduce the candidate population in human identification to save large amounts of time and effort (Heinrich et al., 2018; Choi et al., 2022).

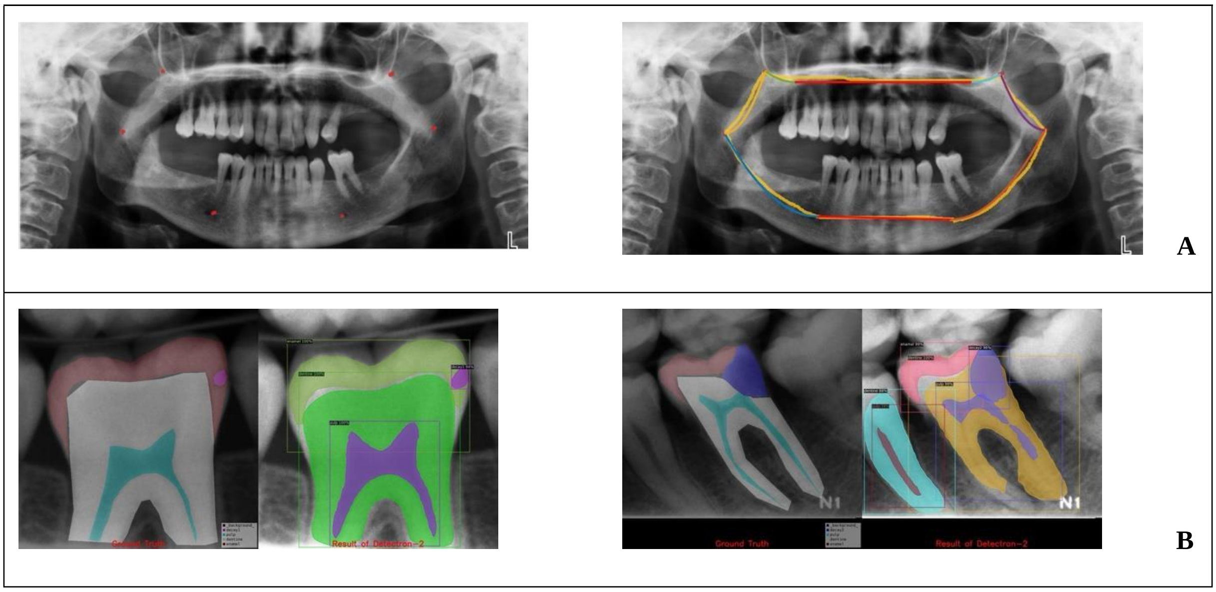


Figure 2: Object detection using deep learning. A. Identification of teeth on dental panoramic radiograph. B. Identification of tooth tissues and caries tissues on radiograph.

Bite mark analysis

Bite mark analysis and data matching in criminal cases can also be done using AI. Bite mark analysis involves detecting, recording, examining and comparing bite marks with existing records. AI can assist in the process by highlighting bite mark images, matching and analysis, and data analysis. It can compare bite marks found on a victim or object with the dental records of a suspect and determine a positive or exclusive match. AI can automate certain tasks, which speeds up the identification process, has a positive impact on accuracy and reduces the possibility of human error (Rodrigo and Baquedano, 2018; Cifuentes-Alcobendas and Domínguez-Rodrigo, 2019; Franco et al., 2019; Vodanović et al., 2023).

Age determination

AI is a tool that can help determination the age and gender of people. Age determination, which is of critical importance for forensic medicine and dentistry, depends on the experience of the specialist, among other factors. Unfortunately, an ideal, fully functional method for age determination has not yet been developed. For children, age is determined by anthropological and dental methods. On the other hand, for adults, the process is primarily based on

degenerative changes and is less accurate than for children. Since age determination becomes less accurate with aging, different methods have been developed to accurately determine adult age (Vodanović et al., 2023; Murray et al., 2024).

The characteristics of an ideal age determination or determination method include being user-friendly and cost-effective, accurate and reliable, not being intervenable, inclusive, acceptable, confidential, and providing fast results. Essentially, the process should produce results that are consistent and accurate with the true age of the person being studied, be consistent regardless of the researcher and the person being studied or the circumstances, and not vary significantly from one measurement to the next. The method used should be easy, should not require rigorous training or special equipment, and should be able to estimate the age of people across races and ethnicities for different age groups. The method used should protect the privacy of the individual and should not disclose personal information beyond age (Marconi et al., 2022; Vodanović et al., 2023; Singh, Singha, and Kumar, 2024).

AI can also be used to estimate a person's age by analyzing images of teeth or jaw bones. Using machine learning methods, dental conditions and characteristics associated with different ages can be identified, and this information can then be used to estimate the age of an unidentified person. AI can be trained to estimate the age of a person based on the development of teeth and their wear to determine chronological age. In fact, it has been found that the accuracy of age determination by AI methods based on Cameriere's stages of dental development is higher than the Cameriere formula. The study results show that machine learning algorithms can be better than the traditional Cameriere formula (Cameriere et al., 2015; Štepanovský, Ibrová, Buk, and Velemínská, 2017; Shen et al., 2021).

Age determination is a process where highly complex methods alone cannot provide accurate results. Therefore, in order to obtain the most accurate age determination, AI should be used along with various methods. Some studies have shown how interpretable Machine Learning and Deep Learning models can be applied to estimate age using root lengths of

second and third molars. AI can also be used in age determination processes to automate some tasks. (Mohammad, Ahmad, Kurniawan, and Yusof, 2022; Wu et al., 2022; Vodanović et al., 2023; Patil et al., 2023).

Sex determination

Sex estimation is an important aspect of forensic sciences as estimating the sex of a victim or suspect can assist with identifying victims of mass disasters, missing persons cases, genealogical research, and sex determination. Sex determination while performing an autopsy also contributes to determining the cause of death by leading to further information about the anatomy and physiology. Therefore, it can be said that the accuracy of sex determination is highly important to eliminate bias or errors in forensic medicine. Depending on the preservation state of tissues, different factors can be used to determine the sex in forensic sciences. As an example, examination of external genital organs can be helpful for the determination process in the presence of well-preserved tissue. However, if it is not possible to determine by external genitals, other parameters such as skeletal structure, cranial measurements, and tooth characteristics can also be used. In those cases, the shape and size of bones such as the pelvis, the size of the forehead, chin and brow ridge, and shape and eruption differences of teeth between males and females can assist in determining sex. Moreover, DNA analysis, in which the presence of the Y chromosome is analyzed is an accurate technique of estimating the sex of an individual, while hormonal variations such as testosterone and estrogen can also be helpful to determine sex. The accuracy of this process is influenced by the variation in methods used and the developmental stage of the individual being examined. Several methods are often used to minimize these challenges and errors. In forensic dentistry, determining sex in children is challenging or nearly impossible because their developmental stage, lacking fully developed sexual characteristics, poses significant obstacles to accurate analysis (Sing, Singha, and Kumar, 2024).

The AI system can be further advanced to recognize patterns and characteristics that differentiate between genders, thereby aiding in forensic sex determination. AI and

neural networks can be used to determine gender by analyzing the size, shape and development of teeth and jaws. AI can also be used to analyze the skeleton to evaluate images such as X-rays or CT to determine sex through differences of size, shape and developmental state (Vodanović et al., 2023).

Facial reconstruction

Facial structure results from a complex combination of genetic, hormonal and environmental factors that is not only affected by growth patterns and genetic inheritance but also arranged by the effects of age, gender and population history. Dietary habits, muscular activity and social trends also contribute significantly to individual facial features (Lieberman, McBratney, and Krovitz, 2002; Evison et al., 2010). Facial features (i.e. eyes, ears, nose and mouth) are crucial for face recognition. The impact of population proximity, age and gender-dependent differences should also be integrated into guidelines to efficiently estimate facial morphology between various demographic groups. These factors also show the need for personalized assessments to reduce errors.

To summarize, it can be said that these predictive models lead to advances in facial prediction through improved soft tissue understanding while assisting with demographic differences in facial morphology, which consequently improves forensic anthropology and outcomes. In many forensic laboratories, unidentified remains are still being discovered each year, creating challenges due to the lack of medical or dental records, fingerprints, DNA and personal documentation. Using digital technology, the Forensic Facial Approach (FFA) provides a promising tool for identification in this context, reconstructing facial features using skull surface analysis and modeling, and improving recognition and potential identity. FFA represents the practical application of anatomy and forensic anthropology. Quantitative studies have aimed to infer population characteristics for anthropological and forensic applications by examining ear morphology, including dimensions, proportions and bilateral irregularities (Sforza et al., 2009). Predictive models for ear shape and dimensions have been developed for FFA (Sforza et al., 2009), showing metric variation

between individuals and populations, with larger parameters and bilateral asymmetry observed in males. The interpretation of oral morphology takes into account tooth alignment, tooth patterns and facial profile. The relationship of ear shape to skeletal structure lacks a comprehensive study, and AI can be used to create 3D models of teeth and jaws for use in the facial reconstruction of unidentified remains (Vodanović et al., 2023; Mbonani, Abbè, and Ridel, 2024).

Investigation of the crime scene

The Metaverse is a digital platform where people can experience real-life feelings through digitally constituted visuals. Users can explore and move the object shown or felt as part of the digital environment. The Metaverse is viewed as the next significant advancement, offering promising potential in global technology and business sectors. Many global companies have already started or are considering investing in the Metaverse. However, as the Metaverse becomes more like the real world, the likelihood of digital events such as money laundering, virtual theft, virtual robbery and fraud emerging as new areas of crime increases. Therefore, the need for digital forensic investigation should also be considered. Although Metaverse holds the potential to become a threat to crimes, developments, like digitally constructing events or crime scenes, may also contribute to forensics (Vodanović et al., 2023; Seo, Seok, and Lee, 2023). Of course, the cost-benefit calculation of each technology should not be one-sided. In this context, simulating a real crime scene in the Metaverse and simulating different scenarios will allow us to examine many possibilities (Figure 3).

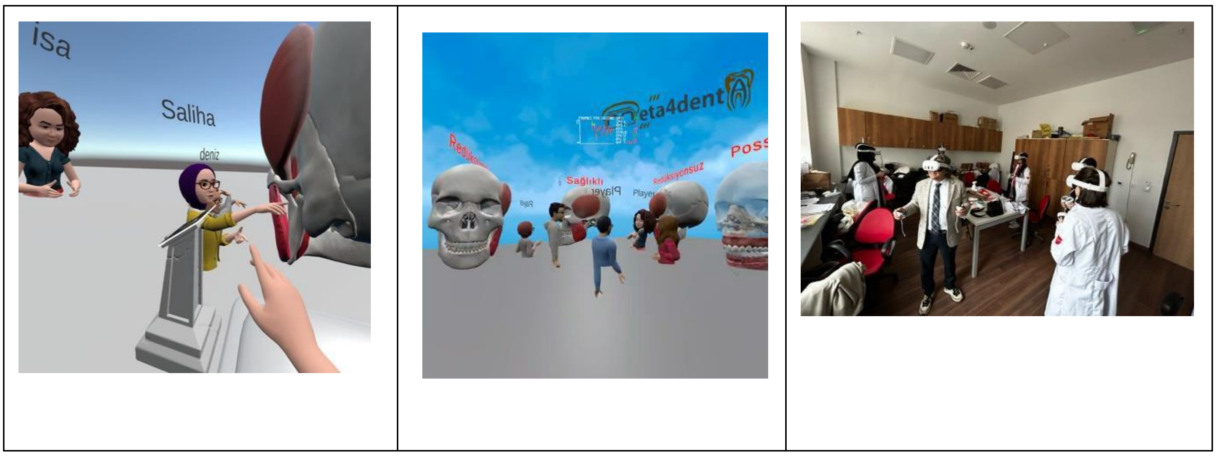


Figure 3: Example of metaverse application in the dental field.

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EVALUATING OF STRATEGIES FOR DETECTING DRUGS IN IMPREGNATED MATERIALS CURRENT AND FUTURE TRENDS

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Chapter 6

Evaluating of Strategies For Detecting Drugs In Impregnated Materials Current And Future Trends

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Introduction

The 2021 European Drug Report reported no decline in the supply of narcotics and large seizures of narcotics in European ports in 2020 and early 2021 (European Monitoring Center for Drugs and Drug Addiction, 2021). One concerning aspect associated with the pandemic is the rise in the accessibility and consumption of medications. For example, between 2009 and 2019, the number of seizures of cocaine (+27%) increased, but at a slower pace than the quantities seized. This probably reflects an expansion of production and transit activities and an increase in domestic consumer markets for drugs. Such a diversity of drug trafficking routes and methods is among the main challenges facing law enforcement agencies.

Due to Türkiye's strategic location for the transfer of narcotics and the strict controls at customs and on the highways within the country, criminal organizations in Türkiye have developed methods for the transfer of illicit drugs such as coffee beans, capsules that are inserted into human and animal bodies (Hergan et al., 2004) or liquefied and impregnated into textiles such as towels, bathrobes, T- shirts, pique, lace and paper (Grabherr et al., 2008). Similar to other countries, in Türkiye, especially during cocaine trafficking inspections, unusual situations, such as 8 kg of cocaine impregnated in 52 kg of clothes, can be encountered.

The illegal drug trade is a worldwide black market focused on the cultivation, manufacturing, distribution, and sale of prohibited substances (Ali & Edwards, 2014). As drug trafficking regulations intensify in numerous nations, traffickers are devising increasingly

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innovative methods to transport their illicit narcotics beyond the reach of law enforcement. Trafficking methods include hiding drugs in a vehicle, luggage, or clothing, impregnating them with any material, or eviscerating a corpse and using it as a storage package (body wrap).

In the international context, drug traffickers are highly creative in concealing narcotics to avoid detection. The literature reports numerous cases where narcotic substances have been mixed with copper, iron, graphite, and coal (Krebs et al., 2000; Laussmann et al., 2015), chemically masked within thiocyanate complexes (Laussmann et al., 2015), impregnated into clothing (McDermott & Power, 2005), absorbed into the cardboard used in boxes transporting fruits and vegetables (Extance, 2020), hidden inside plastic (Gostič & Klemenc, 2007), dissolved in liquids (Burnett et al., 2011; Gambarota et al., 2011), or dispersed in polyvinyl alcohol (van Nuijs et al., 2012). Other instances include concealment within wax (Jellema, 2001), book covers, baseball caps (Drug Enforcement Administration, 2003a), wooden spheres (Drug Enforcement Administration, 2003b), beer cans, milk cartons, bamboo sticks, wicker baskets, or dissolved in alcoholic beverages such as wine and hidden in wine bottles (Grabherr et al., 2008). These cases illustrate various creative methods used in drug smuggling operations.

Psychotropic substances, which have been illegally smuggled into prisons in recent years, are a vital security problem (Ford & Berg, 2018). In a study in which letters sent to prisoners in United Kingdom (UK) prisons were analyzed by UPLC-MS/TOF, it was reported that psychotropic substances were detected in the letters. The identified chemicals comprised the stimulants ethylphenidate, metiopropamine, and methoxyphenidine; the sedative etizolam; and the third-generation synthetic cannabinoids 5F-AKB-48, AB-FUBINACA, and MDMB-CHMICA. Additional chemicals identified included the class A drug cocaine, the class B drug methylphenidate, as well as lignocaine, benzocaine, and procaine. There is no data on the quantitative results of the substances detected in the study and the extent to which the ink and dyes on the drug-impregnated paper affect the concentration of drugs in the envelopes containing

manuscripts, letters containing poetry, and children's drawings.

1. New Trend Analysis Techniques

In recent years, a new trend in illicit drug trafficking has been identified in the form of "mixtures of narcotics and polymers" disguised on fabric to avoid detection and identification (Akhtar et al., 2022). This renders drug detection at checkpoints, such as airports, nearly unfeasible using handheld instruments employed by anti-narcotics personnel and customs departments. Since the detection and identification of polymer-bound narcotics is difficult with today's technology, methods need to be developed to address this.

A trend in drug trafficking is emerging involving narcotics that are typically sprayed or infused onto paper (Extance, 2020). Raman spectroscopy is frequently favored for the detection of drugs. A significant restriction of Raman spectroscopy for drug detection, alongside its advantages, is its sensitivity (Kranenburg et al., 2021). The analysis of substances can be challenging due to the impurity of drug-containing samples, as the evaluated signal incorporates characteristics from other compounds that may confound database searches. Moreover, the existence of fluorescent dyes or contaminants in a sample, even at minimal concentrations, might obscure Raman signals and hinder the identification of the target chemical.

Law enforcement and customs services frequently rely on color tests as the primary screening instruments for seized samples when expeditiously determining whether to detain an individual or to authorize the release of a shipment to a cargo carrier, including during nighttime and weekends. This preference is attributed to their ease of use, cost-effectiveness, and rapid result generation, typically within a few seconds (de Jong et al., 2018; J. Eliaerts et al., 2021). The identification of narcotics confiscated during routine highway inspections at airlines, airports, customs, or customs offices is conducted using color tests. Notwithstanding its drawbacks, the Scott color test remains the most prevalent field test for cocaine detection (Tsujikawa et al., 2017).

An alternative rapid screening method to these color tests is Mid Infrared (MIR) spectroscopy combined with chemometric techniques (Joy

Eliaerts et al., 2017). Traffickers often process drug powders in tinted forms to avoid the detection of powdered drugs by such tests and to mask the positive results of on-site color tests (Eliaerts et al., 2021). In a study in which brown, black, red, and green colored powders were found packed in suitcases during routine checks, the seized powders were first analyzed by an on-site color test, and a negative test result was obtained. The MIR spectra obtained by MIR, when compared with the reference library, showed negative matches with cocaine and positive matches with substances such as propyl benzoate, cellulose, ammonia, potassium permanganate, and aluminum silicate. Subsequently, the content of these powders analyzed by GC-MS was decided to be cocaine, and 8%-35% of the total weight of the powders was determined to be cocaine by quantitative analysis. The results of other screening tests for cocaine, such as on-site or color tests and MIR spectroscopy, are only hypothetical and require confirmation by advanced analytical methods such as chromatographic techniques.

Narcotic substance detection conventionally employs immunological-based colorimetric tests, Scanning Electron Microscopy-Energy Dispersive X-ray Spectroscopy (SEM-EDX), Mid-Infrared (MIR) spectroscopy, handheld Raman spectroscopy, and confirmatory techniques such as High-Performance Liquid Chromatography (HPLC), Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS), Gas Chromatography-Mass Spectrometry (GC-MS), Fourier Transform Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance Spectroscopy (NMR), Gas Chromatography-Flame Ionization Detector (GC-FID), Raman spectroscopy, Attenuated Total Reflectance-Mid Infrared (ATR-MIR), Diffuse Reflectance-Near Infrared (DR-NIR), and analogous analytical methods (de Oliveira Penido et al., 2016; Eliaerts et al., 2021; Pérez-Alfonso et al., 2018; Xiao et al., 2018). Moreover, contemporary techniques like Fiber Spray Ionization Mass Spectrometry (FSI-MS) have been utilized for the detection of narcotic substances in diverse matrices, including biological samples (Filho et al., 2020), while portable NIR spectroscopy is employed to furnish qualitative or quantitative data (Coppey et al., 2020).

Any sample found positive by prior testing should be subjected to confirmatory testing (Franck et al., 2019). Samples coming to the forensic chemistry/toxicology laboratory for the detection of psychoactive substances are usually separated and identified by TLC, GC, HPLC, and LC-based chromatographic methods. In addition, substance groups can be identified by comparing them with licensed data libraries in analytical devices (Norman et al., 2020). Due to their high selectivity, samples subjected to pre-purification processes, such as extraction, are then validated and qualitatively or quantitatively analyzed with analytical systems, such as HPLC, GC-MS, LC-MS, and LC-MS/MS. Functional groups of narcotic substances are determined by FTIR spectroscopy. With GC-MS, main group determination is performed using substance-specific analysis methods. The continuously updated library of GC/MS and its ability to simultaneously separate and identify many substances in mixtures make this technique a good choice for the identification of narcotics obtained by impregnation (Wang et al., 2006). With LC-MS/MS, the molecular weight of the compounds is determined, and identification can be performed (Norman et al., 2020). With the HPLC technique, screening tests of substances are performed, and purity rates can be determined as well as quantification. Although the Gas Chromatography-Flame Ionization Detector (GC-FID) lacks the superior sensitivity and specificity of GC-MS, it is a simple, reproducible method with a wide linearity range. Therefore, it is occasionally preferred by laboratories with limited resources for the detection and confirmation of narcotic substances on drug-impregnated materials (Akhtar et al., 2022). The only limitation of the GC-MS technique is that the substance to be analyzed must be either volatile or volatilized (Wang et al., 2006).

In contrast, LC-MS/MS can analyze polar, volatile/non-volatile, temperature-degradable/non-degradable compounds that generally cannot be analyzed by GC-MS (Elian et al., 2011). GC/MS and LC-MS/MS (especially LC-QToF and LC-LTQ Orbitrap) techniques are preferred, especially for the identification of substance groups, such as new-generation synthetic cannabinoids and synthetic cathinones (Norman et al., 2020; Akca et al., 2024). LC-QToF and

LC-LTQ Orbitrap techniques are effective analytical methods for simultaneously determining psychoactive substances with high diagnostic accuracy, suitable for confirmation and determination since they can give the mass number up to 4 digits after the comma.

Nuclear magnetic resonance (NMR) spectroscopy is a very adaptable analytical technique used to get precise information regarding the structure, dynamics, and interactions of organic and inorganic compounds (Qriouet et al., 2019). NMR spectroscopy is the method of choice for unambiguously determining the stereochemistry and structure of compounds. NMR spectra enable rapid identification of analytes in detecting psychoactive substances from samples. Since the NMR method is non-destructive and allows subsequent re-analysis of the sample by other analytical methods, it is often preferred in forensic chemistry analysis. Direct NMR spectroscopy (without chromatographic separation) is applicable for the identification and quantification of pharmaceuticals, narcotic agents, or novel synthetic cannabinoids across various matrices (Vajs et al., 2018).

Raman spectroscopy (RS) is a vibrational spectroscopy method associated with inelastic scattering arising from rotational and vibrational transitions within a molecular framework (Khandasammy et al., 2018). Raman spectroscopy is acknowledged as the most discerning spectroscopic method and possesses significant potential for several applications, including forensic science. RS can be efficiently utilized across several domains due to its capacity for speedy analysis, minimal analytical costs, and the absence of any pre-treatment that could potentially harm the substance under examination. Notwithstanding the technological benefits of RS, data processing is more complex than those of standard analytical methods, necessitating chemometric expertise for signal interpretation.

Standoff detection using RS is a topic that has been studied in recent years (Khandasammy et al., 2018). Remote detection is based on the principle of remote analysis of unknown substances to ensure the safety of scientists, technicians, and law enforcement agencies. Using portable RS, drugs can be detected on objects at

airports, coast guard points, ports, prisons, etc. (Vandenabeele et al., 2014). Portable handheld Raman devices can measure at detection limits as sensitive and reliable as an analytical instrument in the laboratory. However, due to existing limitations in the design of portable RSs, field analysis can be problematic due to fluorescence-induced interference from drug particles in contaminated clothing. However, a prototype portable RS operating at 1064 nm (DeltaNu Advantage unit) overcomes many of these drawbacks and problems with the advantage of using long wavelength excitation. In their study, Hargreaves et al. detected psychoactive substances, such as cocaine hydrochloride (Coke), methylenedioxymethamphetamine (MDMA, Ecstasy, Big E), and amphetamine sulfate (Speed) from pure samples or adulterated samples using a portable handheld RS device on objects in an airport environment (Hargreaves et al., 2009). In this study, *DeltaNu Inspector Raman* and *Renishaw RX210 (RIAS)* instruments were deployed in the Terminal 2 arrival area of London Gatwick Airport, and results were obtained in less than 30 seconds per sample for analysis. Thanks to the drug library stored in the instrument, which can be updated frequently, it was observed that the peaks of the reference standards stored in this library matched the peaks of the samples exactly. One of the recent developments in RS is the study (Antonides et al., 2019), which shows that the second wavelength gives better results in narcotic detection in handheld Raman devices operating at 785 and 1064 nm wavelengths. In particular, it was reported that fewer false positives or false negatives were obtained in new-generation psychoactive substance screening at this wavelength.

Portable near-infrared (NIR) technology, widely utilized in pharmaceutical quality control, is favored for creating a rapid and portable analytical method to yield prompt and dependable results from confiscated materials, particularly for on-site analysis, whether conducted immediately at the location or in transit (Coppey et al., 2020). This technology is also used to analyze adulterated pharmaceuticals and identify and quantify illicit drugs.

Ion Mobility Spectrometry (IMS) is a widely used trace detection device for detecting particles and their presence at

checkpoints (Gent et al., 2021). The IMS system is used for particle analysis by zooming in on objects, as well as for the detection of airborne particles. Systems with higher sensitivity can be developed by adding various modules to the IMS device. The *IONSCAN-LS IMS* analyzer, developed as an example, is a preferred analytical system due to its simplicity of use, high sensitivity, speed of operation, and efficiency in sample collection. Synthetic cannabinoids often enter prisons or rehabilitation centers on impregnated papers/cards and through the mail (Ralphs et al., 2017; Ford et al., 2018; Caterino et al., 2019). It has been reported that synthetic cannabinoid- impregnated letters, leaves in books or drawings of children have been detected in various prisons in Finland, Germany, Hungary, Lithuania, Poland, Sweden, the United Kingdom, and the United States (Norman et al., 2020; Akca et al., 2024). IMS, a preferred technique for trace analysis of illicit drugs at security points, such as airports, is now frequently used to rapidly screen all materials entering prisons (especially synthetic cannabinoid receptor agonists in infused paper) (Metternich et al., 2019).

Although the libraries of IMSs are limited to classical drugs, the libraries of these systems are being updated daily, especially with research on the detection of synthetic cannabinoids from different material surfaces (different quality paper surfaces, cosmetic products, food samples, etc.). In addition, overdose poisonings and deaths in prisons as a result of intoxication with synthetic cannabinoids, which people with an addiction use to escape the monotony of prisons, are also noteworthy (European Monitoring Centre for Drugs and Drug Addiction, 2018). Due to concerns about synthetic cannabinoids being impregnated in incoming mail, some prisons have changed their administrative policies only to allow inmates to receive photocopies of mailed items (Hvozdovich et al., 2020). Immunoassays are based on the basic principle of microparticle capture analysis. It is one of the most commonly used methods for detecting psychoactive substances (Hoffman et al., 2016). In these analysis setups, the psychoactive substance to be searched for in the sample is usually collected in a certain region and at a high concentration, and visible and colored

bands are formed. The surface used for analysis usually consists of a nitrocellulose membrane. The structure of these tests includes a colored and microparticulate antibody to the psychoactive substance and a collection zone consisting of a steady-state substance. These are commercial kits based on a presence/absence analysis in the form of a cassette, in which a sample in any liquid form is run in a cassette containing a matrix or strip, and within minutes, a positive or negative result is observed according to the presence or absence of a colored band.

Proton Transfer Reaction Mass Spectrometry (PTR-MS) is a system with the potential to detect particles on living or inanimate surfaces with a high level of reliability (Jürschik et al., 2012). Due to the relatively high sensitivity of PTR-MS, there is very little interference between the background noise and the fundamental peaks of protonated matter. Therefore, different compounds can be identified at low concentrations.

The ambient Pressure Laser Desorption (APLD) technique has been frequently preferred in on-site analyses for the detection of (il)legal substances in recent years (Reiss et al., 2018). Various modifications have been made in the literature to detect different substances and their derivatives. Since APLD can be used to quickly detect smuggled synthetic drugs from objects in huge containers or impregnated with different objects, it provides great convenience to law enforcement agencies during the investigation phase of the incident.

AI-MS (Ambient Ionization-MS) mass spectrometry, a portable MS system, is a mobile technology that offers field-resistant, on-site tandem MS analysis with increased selectivity for chemical identification (Mulligan et al., 2018). This technology allows for analysis without compromising the authenticity of the evidence and without requiring much preparation. Since the sample preparation step is simple, the analysis can be performed simply and efficiently, even by operators unfamiliar with the technique.

Scanning Electron Microscopy (SEM) is a microscope in which conductive/non-conductive samples are examined at high resolution by applying high current to the samples (Elie et al., 2009). Surface structures can be observed with the secondary electron image (SEI) and compositional

distribution with the backscattered electron image (BEI) in SEM. With SEM-EDS, high-resolution images of objects can be created, point regions on the sample can be analyzed, and three-dimensional surface images can be accessed. Scanning Electron Microscopy (SEM) generates images by detecting secondary or backscattered electrons released from a material's surface as a result of excitation by a source electron beam. In the SEM, the electron beam traverses the sample, while the detectors chart the detected signals according to beam position to create an image. The resolution limit of the scanning electron microscope (SEM) is around 5 nanometers. SEM images depict surface morphology instead of internal structure, enabling the generation of three-dimensional images during scans. Thanks to its EDS feature, it allows both quantitative and qualitative determination of the elemental structure of the objects under investigation. It can also accurately determine nanoscale images of sub-micron-sized evidence and elemental analysis of nanoscale images.

When the statistics related to drug smuggling through impregnation methods are examined, cocaine is observed to be the most frequently encountered substance. A case study in the literature revealed that when the luggage of a woman traveling from South America to the Republic of Ireland was inspected at Dublin Airport, it was found that cocaine constituted approximately 14% of the total weight of her clothing (McDermott & Power, 2005). This analysis indicates that the cocaine extracted from the clothing was in hydrochloride form, with a purity level of around 80%. Upon examination of the clothing under filtered light prior to chromatographic analysis, it was apparent that the liquid containing cocaine was locally impregnated and applied via the pouring method. Berber et al. conducted a study in Türkiye aimed at combating drug abuse by developing methods to counter various smuggling techniques. The analysis of liquid cocaine embedded in towels, bathrobes, and t-shirts was performed using gas chromatography-mass spectrometry (GC-MS) with a solid-liquid extraction method. Berber et al. (2010). The cocaine's purity examined in this investigation was ascertained. Drug-impregnated materials are created by applying drug solutions to the material and for the solvent to

evaporate. (Ali et al., 2010). A study illustrated the application of portable Raman spectroscopy for detecting and identifying cocaine hydrochloride in drug-impregnated textiles, utilizing wool, silk, and cotton as representative natural fibers, and polyester as a representative synthetic fiber. This study utilized blue jeans and an orange t-shirt as exemplars of dyed apparel frequently encountered in daily life. The existence of spectral bands from the fiber polymers and/or dyes did not hinder the identification of cocaine, which was distinctly recognizable by its typical Raman bands. This work, involving solely cocaine, illustrates that Raman spectra of cocaine may be readily acquired in situ in a non-destructive way within 20-60 seconds and without the need for sample preparation. A weakness of the study is the absence of information regarding the impact of the dyes on the recovery of the impregnated cocaine. At Santiago Airport, 29 garments contaminated with MDMA from Spain were confiscated. The National Institute on Drug Abuse states that elevated doses of MDMA can disrupt the body's thermoregulation, resulting in hyperthermia that may damage liver, kidney, or cardiac function, potentially leading to fatality. (Parrott, 2012). The identification of these compounds at customs is crucial, particularly as wearing garments infused with narcotic agents can lead to life-threatening complications.

Molina Moreno et al. (2014) employed fingerprint lifting tape and two colored packing tapes (white and green) to assess the detection likelihood of drug particles gathered with clear and colored adhesive tapes. Moreno et al. (2014). Despite the interference and prolonged analysis time caused by impurities, such as textile fibers and residual fingerprints, the confocal Raman spectra of the particles facilitated the detection of cocaine. A study on the detection of drug and adulterant residues in human fingernails, which may become contaminated through contact with illicit substances present on various surfaces, such as impregnated clothing, evaluates the potential for identifying crystals of cocaine and paracetamol (5- 20 μm in size) on the nail surface using confocal micro-Raman spectrometry (Ali et al., 2008). Reports indicate that drug crystals can be identified behind a coat of red nail lacquer on drug-contaminated nails. Moreover, Spatially

Offset Raman Spectroscopy (SORS) exhibits significant potential for detecting cocaine concealed within transparent glass bottles of alcoholic beverages or within a brown rum bottle. Eliasson et al., 2008. This technology demonstrates the potential for the swift and non-invasive examination of dubious beverages, concurrently enhancing efforts to combat drug trafficking.

Conclusion

Drug use and addiction are increasing in the world and Türkiye every year. Along with this, the number of drug-related judicial incidents is also increasing. One of the most fundamental problems of laboratories dealing with forensic sciences is to give accurate and precise answers to questions, such as what the captured substance is, its degree of purity, and the amount seized as soon as possible. Various studies in the literature determine the degree of purity and concentration of substances with drug properties.

These investigations utilize on-site analyses (de Jong et al., 2018; Eliaerts et al., 2021) and employ Mid-Infrared (MIR) spectroscopy in conjunction with chemometric approaches, serving as a quick screening method alternative to colorimetric tests (Eliaerts et al., 2017). Traditional analytical methods for detecting narcotic substances encompass immunological-based colorimetric tests, Scanning Electron Microscopy-Energy Dispersive X-ray Spectroscopy (SEM-EDX), handheld Raman spectroscopy, and confirmatory techniques such as High-Performance Liquid Chromatography (HPLC), Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS), Gas Chromatography-Mass Spectrometry (GC-MS), Fourier Transform Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance Spectroscopy (NMR), Gas Chromatography-Flame Ionization Detector (GC-FID), Raman spectroscopy, Attenuated Total Reflectance Mid-Infrared (ATR-MIR) Spectroscopy, Chemometrics, and Deep Red/Near Infrared Emission (DR-NIR) (de Oliveira Penido et al., 2016; Eliaerts et al., 2021; Pérez-Alfonso et al., 2018; Xiao et al., 2018). In addition to these techniques, Fiber Spray Ionization Mass Spectrometry (FSI-MS) has recently been employed for the analysis of

narcotic substances across various matrices, including biological samples (Filho et al., 2020), alongside portable Near Infrared (NIR) Spectroscopy for delivering qualitative or quantitative data (Coppey et al., 2020). In recent years, the detection of psychotropic substances impregnated in letters sent to inmates, posing a significant security issue, has been achieved using UPLC-MS/TOF (Ford & Berg, 2018). Moreover, the identification of residues from narcotic and adulterant chemicals on human nails, which may get contaminated by the handling or misuse of illegal substances, has been conducted utilizing Confocal Micro-Raman Spectroscopy (Ali et al., 2008). Additionally, Spatially Offset Raman Spectroscopy (SORS) has been employed to identify narcotics concealed within transparent glass containers of alcoholic beverages or inside a brown rum bottle (Eliasson et al., 2008). In cases where multiple narcotic substances are impregnated in mixtures, chemometric estimation of the quantities of components within the mixture can be made using multivariate calibration techniques for quantitative determinations (Popovic et al., 2019).

It is easy to detect the presence of narcotics from drug-impregnated materials, such as clothing, towels, luggage, paper, and cardboard, by various analytical techniques (Alberink et al., 2014). However, determining the total quantity of substances in volumetric and large-scale matrices, such as impregnated materials, can only be achieved by examining the entirety of the material. Depending on laboratory facilities and procedures, this can be time-consuming and expensive, making it difficult or even impossible to examine all of the impregnated material seized. For homogeneous powders and liquids containing drugs, confidence intervals for the total amount of drugs in the powder or liquid can be determined once drug concentrations have been determined since the relevant measurement uncertainty is known based on control chart information. However, this calculation can be more complex because the distribution of drugs impregnated into clothing is not homogeneous. The ISO 17025 standard requires forensic laboratories to determine and report the uncertainty in their measurements (Standard, 2006). The methods described and exemplified in this study

pave the way for the future development, advancement, and application of sensitive and accurate methods for testing drugs and serve as a reference for routine criminal laboratories.

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TRANSNATIONAL STRATEGIES: EVIDENTIAL APPROACHES TO NPS DETECTION IN PRISONS

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Chapter 7

Transnational Strategies: Evidential Approaches to NPS Detection in Prisons

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1. Introduction

In recent years, the illicit drug market has become increasingly dynamic. Traditionally, this market was dominated by classic drugs including amphetamine derivatives, heroin, and cocaine. However, there has been a notable rise in the emergence and distribution of new drugs, collectively referred to as New Psychoactive Substances (NPS), which are also known as "designer drugs" or "legal highs." These new substances that are rapidly gaining a presence in the illicit market, present new challenges (Santos et al., 2024).

1.1. Definition and Scope of New Psychoactive Substances (NPS)

The expression "new psychoactive substances" encompasses a variety of substances that fall outside the regulatory framework of the United Nations drug conventions, though some may be controlled at the national level. This category includes stimulants, cannabinoids, opioids, benzodiazepines, dissociatives, and hallucinogens. Many NPS are created to replicate the effects of controlled substances and are marketed as "legal" alternatives. However, they may carry similar health and social risks to those drugs that are subject to international regulation (European Union Drugs Agency [EUDA], 2024b).

NPS are often more affordable and significantly more potent than traditional controlled drugs. Their use spans a wide range of individuals, including recreational users, those seeking self-medication, individuals aiming for performance or appearance enhancement, and vulnerable populations such as the homeless and

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those involved in high-risk drug activities. Furthermore, because NPS can be difficult to detect with standard drug testing methods, they are also favored by individuals subject to frequent testing, including prisoners, participants in drug rehabilitation programs, and drivers (European Monitoring Centre for Drugs and Drug Addiction [EMCDDA], 2021).

1.1.1. Definition of NPS

The United Nations Office on Drugs and Crime (UNODC) defines NPS as "substances of abuse, either in pure form or as part of a preparation, that are not regulated by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances but may still pose a risk to public health" (United Nations Office on Drugs and Crime [UNODC], 2020). As stated by the UNODC, the term "new" does not strictly indicate recent discoveries, as some NPS were originally synthesized decades ago; rather, it refers to drugs that have only lately emerged in the market. In the market, NPS is also known as "legal highs," "bath salts," and "research chemicals." (UNODC 2024f).

The UNODC has classified NPS into 15 groups: aminoindanes, benzodiazepines, fentanyl analogs, lysergamides, nitazenes, phenethylamines, phencyclidine-type substances, phenyldates, piperazines, phenmetrazines, plant-based substances (such as khat and kratom), synthetic cannabinoids (SC), synthetic cathinones, tryptamines, and other drugs (UNODC 2024c).

Similar to many illegal drugs, NPS is consumed in various ways, such as smoking, snorting, oral ingestion, or injection. They may be provided in different forms, like powders, tablets, or liquids (e.g., synthetic cathinones), whereas some are sprayed onto herbal material to resemble conventional herbal materials (e.g., SC). Moreover, regardless of age, socioeconomic status, or geographic location, individuals from diverse demographics engage in their use (Santos et al., 2024).

1.1.2. The rise of NPS use and its emergence as a global issue

NPS has emerged as a global concern, with at least one such substance being reported by 141

countries and regions worldwide. By June 2024, governments, laboratories, and partner organizations had notified the UNODC Early Warning Advisory (EWA) on NPS of a total of 1,239 distinct substances. The NPS currently on the market replicate the effects of internationally regulated drugs, including cannabis, heroin, cocaine, LSD, methamphetamine and MDMA (ecstasy). Examination of the effects of synthetic NPS as of November 2023 reveals that the majority act as stimulants, succeeded by traditional hallucinogens and synthetic cannabinoid receptor agonists (SCRAs). Additionally, there has been a marked rise in the presence of synthetic opioids in recent years (UNODC 2024a, UNODC 2024f).

The health dangers linked to NPS have escalated with the advent of significantly potent drugs that carry a significant risk of accidental overdose and even death. The unpredictable purity and composition of NPS-containing substances pose serious threats to users, as reflected in the growing number of hospital emergency admissions and fatalities associated with their use (UNODC 2020).

The diverse nature, properties, and structure of NPS create serious obstacles for drug users and healthcare professionals, particularly those in addiction treatment, as well as for scientists, forensic toxicologists, healthcare systems, and international drug regulation policies. These substances were called a "rapidly expanding global epidemic." (Shafi et al., 2020).

2. Importance of NPS in Prisons

Substance use is prevalent in correctional facilities and is often associated with heightened violence and negative outcomes after release, including reduced job opportunities. Depressant substances, including cannabis, heroin, and sedatives, are commonly used within prison settings, as they assist inmates manage the harsh conditions by alleviating insomnia and making time feel like it passes more quickly. Stimulants, on the other hand, are more commonly used by the general population for their social benefits, e.g., increased alertness and promoting wakefulness (Norman et al., 2024).

2.1. Current State and Challenges of NPS Use in Prisons

A major contributor to the increase in smuggling and drug use in prisons has been the increased availability of synthetic drugs. In particular, synthetic cannabinoids and cathinones have emerged as pressing issues in the prison drug market due to their unexpected and potentially harmful impacts. Synthetic cannabinoids, and cathinones, and opioid analogs can be converted into liquid form and applied to items like dried plant material or paper, including mail, or concealed within everyday products such as candy or toiletries, facilitating easier smuggling. These substances have been associated with overdoses and fatalities in the prison system (Parsons et al., 2021).

Different smuggling routes are commonly used by enterprises, individual suppliers, and social sharing networks to bring drugs into prisons, with these routes varying depending on the specific context and security measures of each facility. The main methods described in the literature for smuggling include visitors, mail, inmates during intake, remand, or work release, prison staff, and items thrown over the perimeter (Norman, 2023).

Tracking the availabilty and use of NPS within prisons presents distinct challenges. These include the absence of a clear, unified definition of NPS due to encompassing a broad variety of substances, the constant influx of new substances each year, and variations in national laws and regulations. In addition, self-report methods are less dependable for NPS because users often do not know exactly what substances they are using. NPS are also chemically diverse, making analytical detection difficult, which can lead to them being overlooked or under-reported (EMCDDA 2018).

2.2. Impact of NPS on Inmates and Associated Security Threats

Drug misuse poses a significant risk to prison security, inmate health and staff safety, while its impact can reach to prisoners' friends, families and wider communities. The presence of drugs undermines institutional security, with research indicating a cyclical relationship between drug-related

problems and criminal behavior. (Hagan, 2017).

The most commonly used NPS are synthetic cannabinoids (SCs), often referred to by inmates as 'Spice' or 'Mamba,' which were among the original brand names for these substances. The use of NPS poses significant risks to prisoners' mental health, as the diverse range of SC compounds and inconsistent dosages can produce highly unpredictable and severe effects. Mental health issues reported from SC use include depression, panic attacks, anxiety, psychosis, paranoia, hallucinations, delusions, aggression, agitation, depersonalization, violent behavior, self-harm, and suicidal ideation (Moyes, 2018). NPS can also have a significant impact on the prisoners' physical health, with commonly reported symptoms including nausea, weight loss, temporary paralysis, rapid heart rate, excessive sweating loss of appetite, convulsions, vomiting, stomach cramps and muscle twitching, hypertension, seizures, and elevated body temperature (hyperthermia) (Abdulrahim et al., 2015; Macfarlane & Christie, 2015).

The use of NPS can trigger unexpected aggression and violent behavior, creating risks not only for the individual but also for fellow inmates and staff members. This heightened violence may arise either from the effects of NPS intoxication or from conflicts over NPS-related debts, which can lead to increased incidents of bullying and violence within the prison setting (Prisons and Probation Ombudsman [PPO], 2017).

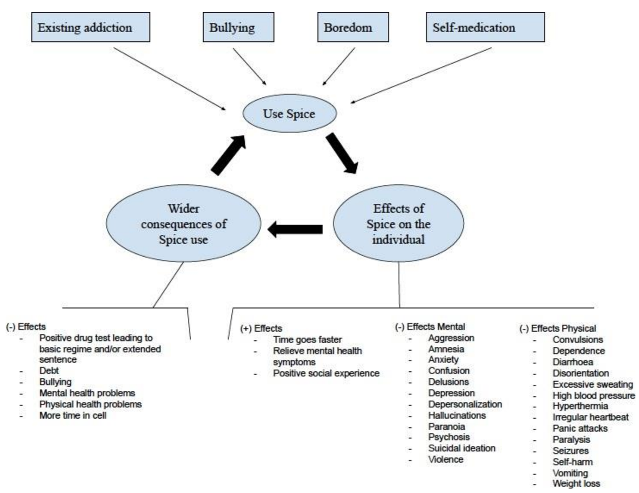

Figure 1. Spice spiral

Violence can lead to segregation or basic regime, which limits interaction with other inmates and the outside world and results in the lack of privileges such as television. For inmates with limited coping abilities, restricted access to social interactions and services may worsen their problems, potentially leading them to turn to synthetic cannabinoids (SC) as a means of coping (User Voice, 2016), thus perpetuating this harmful cycle, often referred to as the 'Spice Spiral,' as illustrated in the figure.

2.3. The Significance of Controlling NPS Use in Correctional Facilities

Effective control of NPS in prisons is crucial for several reasons. First, the widespread availability and use of NPS can destabilize the prison environment, undermining security measures and contributing to a rise in violence, bullying, and illicit trade (Ralphs et al., 2017).

Controlling NPS use is crucial not only for ensuring the safety and security of inmates and staff but also for maintaining the overall stability of the prison environment. One of the major concerns is that NPS use undermines rehabilitation efforts. The mental health effects of NPS, which include paranoia, hallucinations, and suicidal thoughts, can disrupt inmates' ability to participate in programs aimed at supporting their reintegration into society. Furthermore, the physical health risks, such as seizures and cardiovascular problems, add a considerable burden on prison healthcare systems, which are already struggling to provide adequate care (Macfarlane & Christie, 2015).

Lastly, the presence of NPS in correctional facilities strains prison staff and healthcare systems. The unpredictability of these substances makes it challenging for medical professionals to provide timely and effective care. Prison staff, meanwhile, must deal with the heightened risk of violence and aggression, often in an already demanding and high-pressure environment (Ralphs et al., 2017).

In conclusion, addressing NPS use in correctional facilities requires a comprehensive approach involving stringent regulation, education for inmates and staff, and enhanced monitoring mechanisms. By controlling the spread and use of NPS, prisons can

not only improve safety but also support the long-term rehabilitation of inmates.

3. International Strategies

NPS pose a significant challenge at both national and international levels due to their rapidly evolving nature. NPS include synthetic drugs mimicing the effects of conventional illicit substances, making them difficult to regulate and control. Below are some key strategies adopted at international levels to address the issue:

3.1. International Approaches

3.1.1. Policies of the United Nations and other international organizations

UNODC and International Treaties:

UNODC leads global efforts to control NPS through its **SMART Forensics**, which helps countries identify and respond to emerging trends. SMART Forensics offers science- driven capacity-building assistance to member states, addressing drug-related challenges. This support ranges from drug detection and analysis to providing early warnings on emerging drug threats and ensuring the safe disposal of seized drugs and chemicals. Through its early warning system, SMART Forensics identifies and communicates new global drug threats to relevant stakeholders across the world. As with the previous Global SMART Programme, SMART Forensics is operated by the Laboratory and Scientific Services (UNODC 2024d).

- Countries are encouraged to adhere to international drug control conventions, including the **Single Convention on Narcotic Drugs (1961)** and the **Convention on Psychotropic Substances (1971)** (The Single Convention on Narcotic Drugs, 1961; Convention on Psychotropic Substances, 1971). These treaties provide the framework for countries to

schedule and regulate substances, including NPS.

International Cooperation and Information Sharing:

- **The Early Warning Advisory (EWA)** platform by UNODC facilitates real-time information sharing on NPS, allowing countries to stay informed on new and emerging substances (UNODC 2024b). In June 2013, the UNODC Early Warning Advisory (EWA) was established to address the global rise of new psychoactive substances (NPS). Its goal is to track, analyze, and report emerging trends related to NPS, providing a foundation for informed, evidence-based policy decisions. Additionally, the EWA functions as a database for NPS information and serves as a platform to offer technical support to Member States.
- The **European Union Drugs Agency (EUDA)** coordinates information exchange between European countries, providing early warnings and data on NPS across the region. EUDA is Europe's foremost authority on illicit drugs, and it is based in Lisbon, Portugal. It offers independent scientific research and analysis on all facets of this evolving threat to individuals and society. Its work supports both EU and national policies aimed at safeguarding European citizens from drug-related harms. On Tuesday, July 2, 2024, the EMCDDA was officially renamed the EUDA (EUDA 2024a).

World Health Organization (WHO) Role:

- WHO evaluates substances for potential scheduling and recommends international control measures. This scientific assessment helps guide global policies on NPS control. WHO assesses the addictive properties and potential health risks of

psychoactive substances. Based on its evaluations, WHO makes recommendations to the UN Secretary-General regarding international regulation under the International Drug Control Conventions, which are then voted on by the United Nations Commission on Narcotic Drugs (CND).

International Narcotics Control Board (INCB)

- INCB is the independent, quasi-judicial body responsible for overseeing the enforcement of the United Nations' international drug control treaties. It was launched in 1968, as per the provisions of the 1961 Single Convention on Narcotic Drugs (International Narcotics Control Board [INCB], 2024). The INCB's Global Rapid Interdiction of Dangerous Substances (GRIDS) Programme is a worldwide initiative designed to assist Member State governments in efficiently reducing the illegal supply of NPS, non-medical synthetic opioids, and associated precursors. Through GRIDS and its earlier projects, real-time information sharing has been enabled via the INCB's exclusive online communication platform, the Project ION Incident Communication System (IONICS), available solely to authorized government users (UNODC 2011).

Interpol and Europol Operations:

- These international law enforcement agencies conduct operations targeting the trafficking of NPS. They facilitate cross-border cooperation and assist national authorities in detecting and dismantling NPS supply chains.

4. National-Level Implementations

National strategies to address NPS usually involve a blend of legislative measures, law enforcement initiatives, public health interventions, and international collaboration. These approaches focus on controlling the supply, curbing demand, and mitigating the harm associated with NPS. Here are some prominent strategies from different countries.

4.1. Examples of National Practices in Different Countries

The International Drug Control Conventions do not monitor NPS, so their legal status varies significantly between countries. By 2022, 67 countries and territories had introduced legal measures to regulate NPS, with some modifying existing laws and others creating new legal frameworks. In regions where a wide variety of NPS have rapidly emerged, many have opted for controls on entire substance groups using a "generic approach" or have enacted analogue legislation, which allows substances not specifically listed to be controlled based on their chemical similarity to those already regulated. At the international level, by March 2023, the Commission on Narcotic Drugs had placed 78 NPS under international regulation. These measures must then be incorporated into each country's national legal system (UNODC 2024f).

In response to the rise of NPS, some countries initially used medicines legislation and consumer protection laws to regulate these substances. Others, considering the unique characteristics and rapid emergence of NPS in their regions, adapted their drug control laws to address the issue. This included measures like listing individual substances, applying generic or analogue controls, and implementing temporary bans or rapid response procedures, sometimes focusing on the effects of NPS. In some cases, countries decided that entirely new laws specifically designed to regulate NPS were necessary (United Nations [UN], 2024).

Various nations have adopted consumer protection laws in different ways. Some, like Poland, have focused on psychoactive substances in general, leading to the widespread closure of "headshops," while others have targeted specific substances. For instance, regulations concerning proper labeling and packaging of goods and food have been applied in Italy to seize herbal products containing synthetic cannabinoids not labeled in Italian. Similarly, the United Kingdom used this approach to prevent the sale of mephedrone under the guise of bath salts or plant-based products. Subsequently, Poland altered its definition of "substitute drug"—initially referring to a drug alternative or a

similar substance—and strengthened health protection laws to regulate such substances if they were suspected of causing harm. Under EU law, a medicinal product does not need to have therapeutic properties, which has allowed this regulation to be applied in controlling NPS. National agencies could demand authorization for its import, supply, or sale by classifying an NPS as a medicinal product. Eight countries employed this strategy to regulate NPS distribution. However, in July 2014, the Court of Justice of the European Union ruled that substances lacking health benefits cannot be classified as medicinal products, limiting the effectiveness of this approach and rendering it impractical for controlling NPS (Varì et al., 2020).

An alternative method for tackling the threat posed by new substances has involved countries regulating them through current drug laws, either by amending or broadening these regulations. As reliable data about new substances is often unavailable, Hungary (2010) and Finland (2011) established scientific risk assessment panels to provide evidence to support control decisions. In order to speed up the legal process, some countries have put in place temporary control measures to allow time to assess whether there is a need for permanent regulation (EMCDDA 2016). For instance, in 2013, temporary control measures were introduced in Latvia and Slovakia, enforced by the Centre for Disease Prevention and Control in Latvia and the Minister of Health in Slovakia. Similar measures for the inclusion of non-therapeutic substances in the lists of controlled drugs were taken in the United Kingdom and Hungary in 2011 and 2012 respectively. These measures, such as temporary orders for certain classes of drugs, allowed authorities to regulate them as controlled substances under drug regulations. In an effort to curb the dissemination of new drugs, Latvia introduced regulatory penalties for personal possession in 2014, while Hungary made possession of more than 10 grams of an active substance illegal. In the same year, the Czech Republic passed a parliamentary law, followed by government decrees, to update its controlled substances list and set a scheduling timeline. Finland also amended its Narcotics Act in 2014 to include banned psychoactive substances and introduced prison

sentences of up to one year for violations related to public health and safety. Several countries, including Luxembourg, Italy, Cyprus, Lithuania, Denmark, France, Norway, Croatia, and Turkey, extended their existing drug laws to cover well-defined groups of substances rather than individual compounds. Finland introduced the term "positional isomers" into its drug laws, while Germany adopted a "group definition" strategy. In contrast, the Netherlands rejected these innovative approaches in 2012 due to concerns that some compounds might have beneficial applications in the pharmaceutical field (Hughes & Blidaru, 2009).

The most comprehensive strategy employed by European countries has been the introduction of new legislation to regulate the illegal distribution of psychoactive substances, as observed in Austria, Ireland, Portugal, Sweden, Romania, and the United Kingdom. Although the development of these laws is quite similar across all the countries except Sweden, there are notable differences. Psychoactive substances are defined as substances that either stimulate or depress the central nervous system in all five countries. In Ireland, Austria, Portugal, and Romania, this definition is linked to dependency, hallucinations, or impairments in motor function or behavior. In contrast, the United Kingdom defines such substances as those that affect a person's mental functioning or emotional state. Both Ireland and Portugal specify that these effects must be 'significant,' while Austria only lists substances that are likely to be abused by certain groups and present a potential risk to consumer health. In Romania and the UK, the substance is not required to be specifically harmful (EMCDDA 2016).

4.2. Legal Regulations and Their Impact on NPS Detection in Prisons

National practices and legal frameworks for detecting and managing NPS in prisons differ between countries but are generally designed to tackle the health and security risks associated with these substances.

Many countries have adopted legislative approaches that allow them to ban entire classes of NPS to avoid the influx of modified substances into prison systems. For example, the United Kingdom's Psychoactive

Substances Act (2016) broadly bans producing, distributing or possessing psychoactive substances within prison settings (Psychoactive Substances Act, 2016).

In the United States, the regulation of NPS is primarily governed by the Controlled Substances Act (CSA). This legislation permits the government to classify synthetic drugs under Schedule I if they are deemed to have a high potential for abuse. Various detection strategies have been implemented, including drug testing and partnerships with forensic laboratories, to combat the use of NPS within the prison system ((Drug Enforcement Administration (DEA), 2018).

In Australia, in March 2015, the Crimes Legislation Amendment (Psychoactive Substances and Other Measures) Act 2015 (Act No. 12 of 2015) introduced modifications to the Criminal Code Act of 1995 and the Customs Act of 1901 concerning psychoactive substances. Due to these amendments, the importation of psychoactive drugs, as defined by the legislation, was prohibited, with specific exemptions for tobacco products and certain goods as prescribed by other regulations. Furthermore, the importation of substances falsely sold as alternatives to serious illicit substances was also banned (Crimes Legislation Amendment (Psychoactive Substances and Other Measures) Act, 2015; UNODC 2024e).

The Irish Criminal Justice (Psychoactive Substances) Act 2010 makes it a criminal offence to advertise, sell, supply, import or export psychoactive substances (unless specifically exempted), if there is knowledge or recklessness regarding their intended human consumption (Criminal Justice Psychoactive Substances Act, 2010). This legislation introduced a comprehensive prohibition on the NPS trade, leading to the shutdown of all 102 head-shops and websites based in Ireland that were distributing NPS. Countries such as Poland and Romania have implemented similar measures (Home Office, 2015). The legislation grants authorities the authority to confiscate and destroy any psychoactive substances, including NPS, that enter prisons. This legislation has helped strengthen enforcement measures within the prison system.

New Zealand was among the first countries to implement proactive legislation addressing the control of NPS. The Psychoactive Substances Act 2013 was a pioneering law aimed at regulating the sale and use of NPS (Psychoactive Substances Act, 2013). Unlike approaches in other countries, this legislation initially permitted the sale of low-risk psychoactive substances, provided they were tested and shown to present minimal harm. However, due to rising concerns over health risks and widespread use in prisons, the law was later amended to impose a blanket ban on all NPS until thorough risk assessments could be conducted (Rychert & Wilkins, 2018).

Germany has taken a strict stance on regulating NPS through the enactment of the New Psychoactive Substances Act (NpSG) in 2016 (New Psychoactive Substances Act (NpSG), 2016). This legislation prohibits entire classes of substances based on their chemical structure rather than targeting specific compounds. By employing this comprehensive legal framework, Germany has been able to address the ongoing alterations in the chemical composition of NPS used in their production (Kühnl et al., 2022).

Countries like New Zealand, Germany, Sweden, Japan, and Canada have established specific legal frameworks to regulate NPS, complemented by the development of sophisticated detection strategies in correctional settings. However, a shared challenge across these nations is the rapid evolution of NPS, which often outpaces both regulatory measures and detection technologies. Countries, such as Germany and Japan, have adopted broad-spectrum legal prohibitions, offering greater flexibility in regulating these substances, though enforcement within prison systems remains problematic. Advanced detection methods have shown potential for identifying NPS but are hindered by high costs and limited scalability. Consequently, ongoing research investment and international cooperation are essential for effectively addressing NPS use in correctional facilities.

5. Techniques for NPS Detection

To detect and identify of NPS present considerable difficulties due to their chemical variability, rapid appearance, and frequent structural alterations. As NPS continue to evolve,

analytical techniques need to be adaptable and highly sensitive in order to identify these compounds across a range of matrices.

5.1. Analytical Methods

Analytical protocols often begin with presumptive detection, a typically qualitative approach, followed by confirmatory testing. These presumptive methods, also known as field tests, are commonly employed for fast and cost-effective analysis in clinical and forensic settings. They provide an indication of the existence or non-existence of a target compound or potential abuse of drug by detecting chemical groups, though they lack the specificity to pinpoint exact substances within those groups. Advantages of these methods include ease of use, as they do not require specialized training, equipment, or extensive sample preparation. Their high sensitivity and portability make them particularly useful for on-site substance detection. Despite their limited accuracy, these tests are widely used in law enforcement due to their practicality. However, after a presumptive test indicates a substance's presence, a confirmatory test is needed to determine the exact chemical nature. Confirmatory methods are more reliable than presumptive tests, but they require advanced analytical instrumentation, expertise, and detailed preparation of the sample, which may be destructive (Bruni et al., 2022).

5.1.1. Laboratory-based detection techniques

For the detection of NPS, various techniques, such as colorimetric tests, immunoassays, and mass spectrometry, can be utilized. Colorimetric assays work by inducing a visible color change when a target analyte reacts with a specific compound. These methods are advantageous due to their ease of use, portability, and suitability for on-site testing, requiring minimal sample preparation. However, limitations include variability in user interpretation of color changes, the potential for cross-reactivity leading to false positives, and a restricted ability to detect multiple NPS compounds in a single sample (Salomone et al., 2016).

Immunoassays provide a means for rapid detection of NPS, offering the

advantage of non-invasive sample testing, such as through urine or drug solutions. Lateral flow immunochromatography has been used in risk mitigation initiatives encouraging opiate users to self-screen for fentanyl. However, market available immunoassays are currently limited to the detection of a restricted range of NPS (Ares et al., 2017).

Gas and liquid chromatography-mass spectrometry (GC-MS and LC-MS) techniques provide highly sensitive and specific methods for identifying individual NPS and quantifying their presence in biological specimens. These methods can be used for a wide range of samples, such as urine, blood, saliva, hair, wastewater, and dried blood spots (DBS). Samples typically require laboratory preparation prior to analysis, although new "Dilute and Shoot" methods are undergoing validation to accelerate sample preparation for LC-MS. Liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (LC-QTOF MS) has shown advantages over GC-MS in detecting various NPS in serum samples. Efforts are ongoing to compile and validate spectral databases for known NPS, which could aid in identifying both known and novel substances based on the analytical method employed (Shafi et al., 2020).

5.1.2. Rapid test kits and portable devices

Many techniques used to identify and quantify drugs in the laboratory are expensive and need specialised staff to manage complex equipment and interpret the data. However, there is increasing interest in developing portable, rapid testing methods that offer the same level of precision and accuracy as laboratory-based systems. These portable technologies are designed to be cost-effective and user-friendly while delivering fast results, making them suitable for use by law enforcement and other field analysts. There are existing and emerging technologies for on-site drug detection and analysis, including presumptive colorimetric tests, portable vibrational spectroscopy devices, point-of-care biosensors, compact ambient mass spectrometers, and other miniaturized analytical instruments (Alonzo et al., 2022).

In certain situations, portable devices are preferred, while in others, although portability is not essential, the

instruments must be compact, lightweight, and durable enough to endure frequent relocations. This is particularly relevant for transporting equipment to festivals or rotating services across multiple overdose prevention sites within a city. Unlike portable devices, laboratory-based instruments tend to be larger and require a fixed, stable location, along with additional site-specific requirements such as high voltage power, ventilation systems, access to compressed gases, and strict control of environmental conditions such as temperature and humidity. Given these logistical challenges, many drug-checking initiatives employ portable instruments, such as infrared (IR) spectrometers, coupled with test strips to facilitate analysis at the site (Gozdzialski et al., 2023).

6. Operational Techniques

Detection strategies in prisons are vital for preventing the smuggling and consumption of illegal drugs, particularly NPS. The prison environment presents specific challenges for identifying these substances, including frequent inmate turnover, varied smuggling techniques, and the fast-paced emergence of new synthetic drugs. To address these issues, prisons generally rely on a combination of advanced technology and manual inspection methods (Inspectorate of Prisons, 2015).

6.1. Operational Detection Methods Used in Prisons

Since the 1990s, many European prisons have implemented drug testing programs, primarily through urine analysis, as measures to reduce drug supply and demand. However, these programs can lead some individuals to shift from using detectable drugs to undetectable ones, such as the rise of NPS in prisons. Additionally, drug testing may have unintended consequences, including raising awareness about the availability and use of drugs. Despite these challenges, data gathered from drug tests can offer valuable epidemiological insights. When combined with other sources of information, like prison surveys, this data can assist create a more thorough understanding of the prevalence and trends of drug use within correctional facilities (EMCDDA 2022).

Prisons commonly employ a range of security measures to prevent visitor-led smuggling, including the use of drug detection dogs, video surveillance, and staff oversight in visitation areas. Certain facilities also perform body searches or use scanners, as mentioned earlier, on both inmates and visitors prior to their entry into visitation rooms. Additionally, items such as clothing brought by visitors may be inspected using X-ray technology. This technology, particularly body scanners, has become a standard tool for point-of-entry screening in prisons and government buildings, proving effective in limiting the smuggling of illicit materials. In the early 2000s, full-body transmission X-ray scanners were initially introduced in correctional settings to reduce the invasive and time-consuming nature of physical searches, particularly when contraband is concealed inside body cavities. Using ion mobility spectrometry (IMS) to screen visitors has proven to be an effective approach for detecting traces of drugs (Norman, 2023; Parsons et al., 2021). IMS is among the most widely used chemical detection tools in correctional facilities due to its user-friendly operation and exceptional sensitivity. IMS is capable of detecting and identifying a broad spectrum of drugs, such as methamphetamines, fentanyl, carfentanil, synthetic cannabinoids, synthetic cathinones, and cocaine (Parsons et al., 2021).

6.2. Use of Trained Personnel and Detection Dogs

In countries like Canada, the United Kingdom, and the United States, detection dogs have been utilized for the detection of both traditional drugs of abuse (TdA) and synthetic cannabinoids (SCs). However, the fast-paced and ever-changing nature of the NPS market. poses challenges in sustaining the long-term efficacy of detection dogs against these drugs. If samples are found to contain drugs, they are analysed using on-site analytical methods such as IMS or sent to external forensic laboratories for confirmatory testing (Vaccaro et al., 2022).

Prison detection strategies are constantly adapting to meet the increasing challenges posed by illicit drug use and smuggling, especially with the rise of NPS. While traditional techniques such as drug detection dogs

and manual searches remain important, the incorporation of advanced technologies like IMS and body scanners, along with enhanced intelligence operations, has significantly strengthened the capacity of prison authorities to identify and intercept contraband substances.

7. Technical Challenges

7.1. Difficulties in Detecting Newly Emerging Substances

Advancements in modern chemistry and pharmacology have facilitated the creation of life-saving drugs but have also led to the emergence of NPS. These compounds are often produced in unregulated, makeshift laboratories without any form of quality control or governmental oversight, leaving their chemical composition, content, and potential adverse effects largely unknown. One of the key challenges in timely detection stems from the rapid turnover of these substances—by the time thorough biochemical and toxicity evaluations are completed, the NPS may have either vanished from circulation or been modified to evade existing regulations. This constant evolution makes detecting and regulating these synthetic drugs particularly difficult (Upadhyay, 2021).

Recently, over 1,000 NPS have surfaced in illicit drug markets. The sheer volume of these substances, combined with the constantly shifting nature of the NPS landscape, poses significant challenges to early detection and the formulation of strategies to mitigate the associated public health risks. This rapid evolution complicates efforts to address the emerging dangers effectively (UNODC 2020).

Traditional drug monitoring systems predominantly target a narrow selection of well-known and banned substances. Forensic and toxicological analytical techniques, depending on the method used, can be applied to detect, identify, and quantify compounds present in confiscated materials or biological samples. However, it is crucial to recognize that conventional methods for addressing illegal drugs may not be suitable for handling NPS. In forensic contexts, precise identification is essential when analyzing collected evidence or assessing cases of suspected intoxication, necessitating the use of

robust and accurate detection methods (Bruni et al., 2022).

From an analytical perspective, accurately identifying NPS presents a significant challenge. The frequent molecular modifications and the rapid pace at which these compounds emerge on the market complicate their evaluation. New substances are introduced faster than analytical methods can be adapted. One of the most pressing issues is the absence of verified reference standards, making accurate identification even more difficult (Peacock et al., 2019).

Similarly, the challenges in detecting SCRAs and other NPS in prison settings likely contribute to their prevalence. Detection dogs are not trained to identify the diverse forms of NPS, and the infusion of these substances into materials such as paper and textiles adds another layer of complexity for prison authorities trying to detect them (EMCDDA 2018).

8. Technological Innovation and Research

8.1. The Impact of Future Technological Advancements on NPS Detection

Analytical procedures typically focus on the targeted detection of a specific set of compounds, which are selected grounded in national or international guidelines or national warning systems alerts. Traditionally, the majority of multicomponent methods used for analyzing NPS have depended on low-resolution tandem mass spectrometry. However, there has been increasing interest in broad-spectrum high-resolution mass spectrometry (HRMS) because of its high mass accuracy, allowing for untargeted screening. Furthermore, advances in structure elucidation of unknown designer drugs, along with novel data analysis and machine learning methods, have enhanced the evaluation of results and the investigation of NPS metabolites (Salomone & Vincenti, 2024).

8.2. Development of More Effective and Cost-efficient Detection Methods

A cost-effective and straightforward method for detecting low concentrations of nitazenes involves the use of a blood microsampling technique called dried blood spots (DBS). Recently, a wide range of novel opioids have been detected by DBS sampling using the UHPLC-MS/MS technique. This method is particularly advantageous for large-scale epidemiological research due to its minimally invasive nature compared to traditional intravenous blood draws. Additionally, DBS offers simplicity in sample collection, transportation, and storage, making it highly suitable for such studies (Salomone & Vincenti, 2024; Ververi et al., 2024).

Conclusion

The rise of NPS in prisons presents a multifaceted threat to both inmate health and prison security. These synthetic drugs, which are often undetectable by traditional means, pose serious challenges for prison authorities attempting to control their spread. As this chapter illustrates, the rapid evolution of NPS requires equally dynamic and innovative detection and management strategies. International cooperation, legal reforms, and technological advancements are essential in addressing the dangers posed by NPS. While countries have adopted various legislative frameworks and detection technologies, there is a clear need for continued research, investment, and the development of adaptable solutions. The global nature of the NPS issue necessitates shared efforts across borders to mitigate the risks associated with these substances in correctional facilities. By improving detection methods and legal frameworks, prisons can not only enhance security but also better support inmate rehabilitation and health outcomes.

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